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## TUBERCULIN SENSITIVITY

In the memorable year 1882, Robert Koch made his epoch-making discovery of the tubercle bacillus. In 1890, he made yet another great discovery—that of tuberculin.

Koch himself demonstrated that while injection of live or dead bacilli in the peritoneum of an uninfected animal caused little reaction, an animal already infected with tubercle bacilli behaved quite differently. In the latter case, a violent inflammatory reaction with peritoneal effusion resulted (Koch's phenomenon). It was soon realised that this hypersensitivity to tuberculo-proteins also follows infection in human beings and appears to persist throughout the life of the individual.

Of the various skin tests devised to elicit hypersensitivity to tuberculosis infection, from the early subcutaneous injection to the latest intradermal injection with jet injector, the intradermal test first proposed by Mantoux in 1908 is the most popular, so much so that tuberculin test is very often referred to as Mantoux test. Mantoux test for detecting presence of tuberculosis infection has been extensively used in epidemiological studies giving us invaluable information about the extent of existing tuberculosis infection and the incidence of fresh infection in many parts of the world.

However, with increasing experience, doubts have started to creep in about the specificity and usefulness of the tuberculin test. In old days when O.T. (old tuberculin) was commonly used, it was advocated that if an individual was insensitive to a low dilution like 1:10,000, tests should be repeated with 1:10,00 and 1:100. Now it is generally recognised that higher strengths of tuberculin will elicit non-specific reactions which are believed to be caused by infection with organisms allied to tubercle bacillus (atypical bacilli or unclassified mycobacteria) which give a low level of reaction with low dilution of tuberculin and stronger reaction with higher strength. Hence most workers agree that the level of reaction to be called specific for tuberculous infection should be at least 15 mm in diameter when 1 T.U. of P.P.D. RT 23 is used. There is sufficient evidence to suggest that children showing 15 mm or more reaction to tuberculin are more likely to develop disease in later life than those showing smaller reaction.

It should, however, be remembered that individuals vary in their reaction when allergic to any substance or infection. The grosser reaction to tuberculin should be taken as excessive allergic response on the part of individual rather than as evidence of more severe tuberculous process,

A paediatrician from the South, having tried different non-official tuberculin available in the market in India has come to the conclusion that most of them are unreliable. He has made a strong plea to liberalise supplies of official tuberculin, namely P.P.D. RT. 23, in order to make correct diagnosis of tuberculous infection in children. With the abolition of the pre-BCG Tuberculin test because of the introduction of direct BCG vaccination, supplies of official tuberculin are held back. We make a strong plea here that PPD RT 23 should be made freely available to Paediatric Clinics as also T.B. Clinics for in the rural areas Tuberculin test is about the only available reliable tool for diagnosis of tuberculosis in children.

Value of tuberculin test in adults has always been limited as the test cannot differentiate between infection and disease. Negative tuberculin test was taken so far as proof of absence of tuberculous disease but even here confusion is caused by the finding that a sizeable percentage (15% to 30%) of proved tuberculosis patients may give a negative reaction.

Later in this issue, an article on "Loss of Tuberculin Sensitivity in Tuberculous Peritonitis" focuses attention on the negative tuberculin test in the presence of tuberculosis infection. There is no adequate explanation as to why the test was temporarily negative in the two cases of peritonitis quoted in the article but there is no doubt that experts should direct attention to research in this respect. Perhaps we will find the answer in the study of the lymphocytes which play an important role in immunity and allergy.

# COST OF ESTABLISHING AND OPERATING A TUBERCULOSIS BACTERIOLOGICAL LABORATORY

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## Introduction

Costing and methods of cost analysis are well known in industry. Their application in the field of health is of recent origin. Under the basic health services a variety of specific health programmes have been introduced to promote health of the community. Health expenditure is generally an investment by the Government and in developing countries with scarce economic resources careful evaluation is necessary in planning, programming and allocating the resources. Health investment is distinct from investment in commercial undertakings because the benefit of the former cannot be easily measured in monetary terms. In addition to the immediate benefit of cure of illness of individuals, manifold long term benefits are also derived by the community. Malaria and Tuberculosis Control Programmes are unequivocal examples where economic benefit accrues to the entire community besides relief of suffering to individuals. Therefore, in implementing such health programmes cost effectiveness should be a major consideration so that services are rendered at as low a cost as possible without sacrificing the quality.

Within the frame of the National Tuberculosis Programme, establishment of a State Tuberculosis Centre in every state is envisaged. The present paper deals with the cost of establishing and running a bacteriological laboratory in these centres. At present, precise information regarding the cost of setting up the laboratory and of various bacteriological examinations is lacking. A knowledge of the costs will enable proper planning and judicious utilisation of the resources. Further, when services are rendered to private individuals or institutions, the charges for different examinations can be levied on a rational basis.

## Objectives

(1) To establish the initial and recurring costs for a laboratory which can handle 12,000 specimens a year, 15% of which are likely to be bacteriologically positive.

(2) To estimate the cost of various bacteriological examinations.

## Methodology

Though the general principles of calculation of costs in any field are the same, the peculiarities of each system have to be well understood before evolving the procedure for calculation. In the case of a bacteriological laboratory costing is done not for any product manufactured but for various examinations undertaken. These can be examinations of specimens by microscopy, culture, sensitivity tests etc. There may be several methods of performing these and it is imperative to lay down the methods to be followed before hand. This will help in considering the cost of the equipment and materials required for the particular methods and also in estimating the work-load for the staff. The number of specimens expected and the number likely to become positive are also important for estimating the work-load.

There are 3 factors which contribute to the cost structure of any manufactured product or services rendered viz., overhead charges, cost of materials and labour. In the case of bacteriological laboratory, in addition, certain essential facilities like cold room, incubator room, gas supply, washing and sterilisation and media preparation are required and the cost of providing these facilities also go to make up the cost of various operations or examinations done. The various factors that contribute to the cost of any bacteriological examination are explained in chart 1.

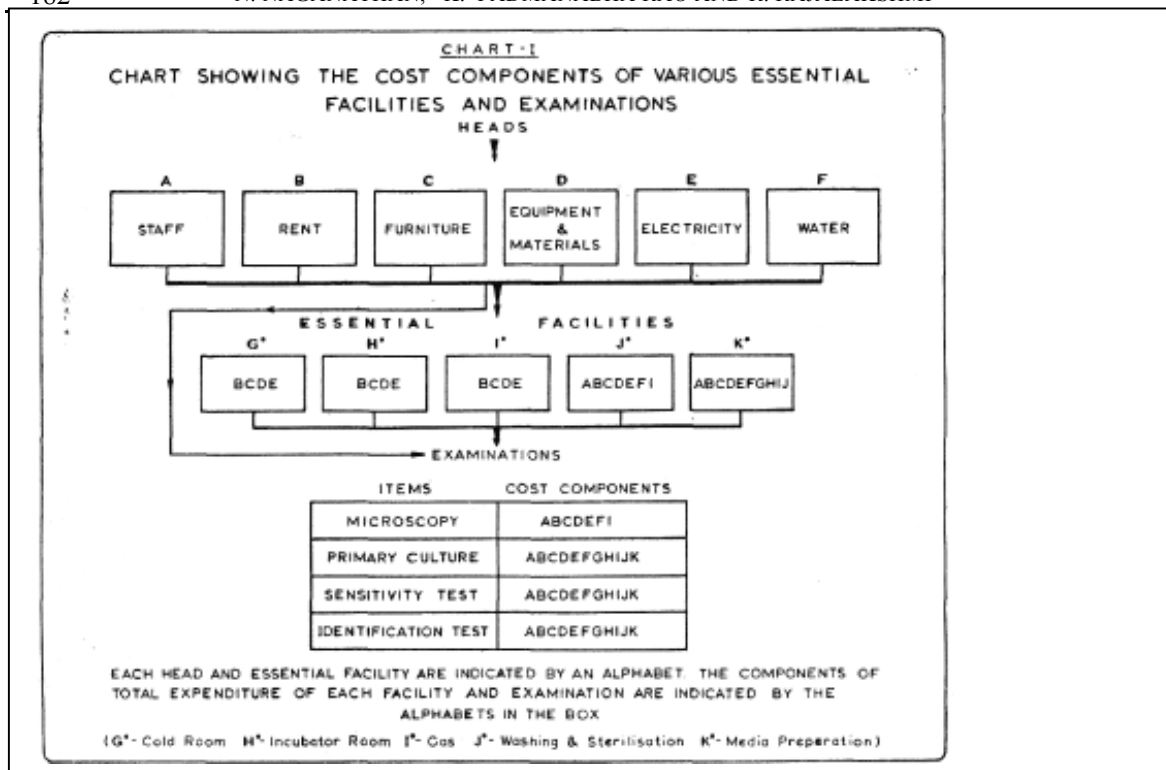
Calculation of cost entails a series of steps and the first step is to list the various bacteriological examinations for which cost has to be worked out. The list is given in table 6.

The second step is to describe the various heads and essential facilities under which expenditure has been incurred. The expenditure on different heads is to be shared by the various facilities and examinations for which the total expenditure has to be worked out. The allocations are shown in table 1. The various heads and essential facilities have been explained in detail below:—

## 1. Heads

### Staff

Each State Centre will have a bacteriologist,



4 laboratory technicians, 3 laboratory attendants and a registration clerk. The pay scales adopted and the total emoluments are as follows:—

that the expenditure on staff will increase year by year because of increase in pay and allowances. The expenditure on

Sl No.	Designation	No.	Scale of pay	Pay	Allowances	Total emoluments for a year
1.	2	3	4	5	6	7
1.	Bacteriologist	1	350-900	350	344	8328
2.	Lab. Technicians	4	100-200	100	70	8160
3.	Lab. Attendants	3	80-110	80	68	5328
4.	Registration Clerk	1	110-180	110	98	2496
Total for the year:						24312

The total expenditure of Rs. 24,312/- on staff is on the basis of the minimum pay drawn by them in their scales. This is done mainly for getting an idea of initial cost of establishing and running a laboratory. It should be remembered

contribution towards pension, provident fund etc., of the staff which should normally be included for costing has been ignored in the present calculation,

Apportioning of expenditure on the staff

TABLE I

*Apportioning of annual expenditure (in rupees) under different heads to various essential facilities and examinations*

Essential facilities and examinations	Establishment	Rent	Electricity	Furniture		Equipment and supplies		Total
				Common	Sectional	Common	Sectional	
1	2	3	4	5	6	7	8	9
Gas plant	—	246	39	—	—	—	555	840
Incubation Room	—	384	133	—	26	—	677	1220
Cold Room	—	877	266	—	49	—	1628	2820
Washing & Sterilization	7885	2350	360	19	134	209	3075	14032
Media preparation	2405	868	266	19	41	309	4998	8906
Sputum smear examination	3698	1270	33	18	66	38	1321	6444
Primary Culture-Concentration Method	5313	603	100	19	36	209	1939	8219
Sensitivity tests	3758	452	100	14	27	51	835	5237
Culture smear	—	—	—	2	6	3	291	302
Identification tests: Niacin	501	50	11	2	3	6	113	686
Catalase & Peroxidase	501	50	11	2	3	6	79	652
Room temperature	251	50	11	2	3	6	31	354
Total	24312	7200	1330	97	394	837	15542	49712

Note : The total expenditure on all with swab method of primary culture is Rs. 49,930.

has been done depending on whether a person has been posted for full time or part time work and the proportionate pay of the individual for a year has been allocated to each examination or essential facility. In the case of bacteriologist and registration clerk, the pay for the approximate number of hours spent on each essential facility and examination has been worked out and allocated and balance of pay has been distributed equitably among all examinations and facilities—washing and sterilisation and media preparation.

#### *Building*

The actual rent of the rented building or the depreciation value plus the annual maintenance expenditure of Centre's own building could be considered for cost calculation. In the present paper a rent of Rs. 600/-per month has been charged. The entire rent has

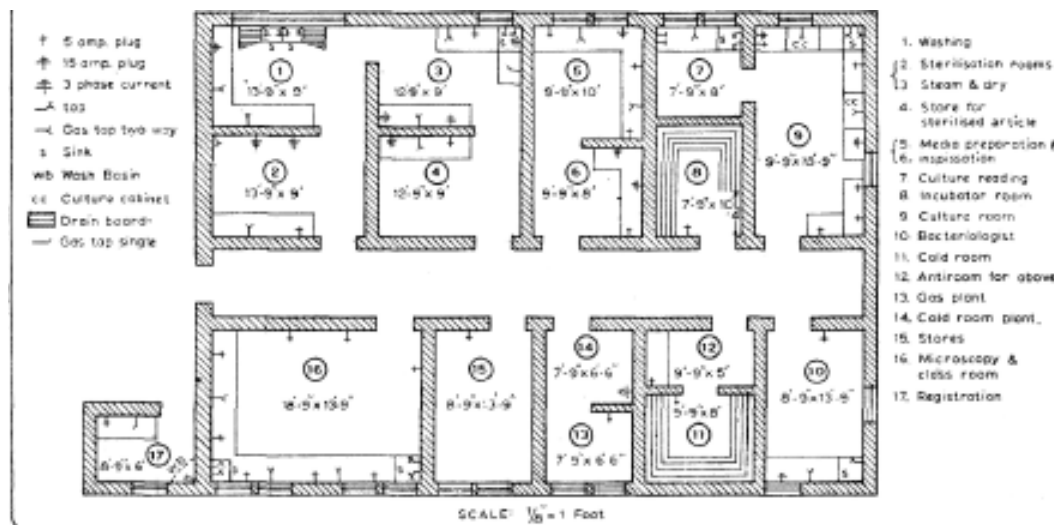
to be shared by different facilities and examinations on the basis of the area occupied by each. Since the area of the corridor, bacteriologist's room, store room, registration room cannot be charged for any examination the total rent is charged for the rest of the area where the actual laboratory work is carried out. The plan and lay-out of the laboratory are shown in Chart. 2.

#### *Electricity*

The expenditure under this head has been calculated on the basis of the electricity charges paid for National Tuberculosis Institute laboratory where on an average 25,000 specimens were dealt with annually. Half the electricity charges of NTI laboratory have been taken for the State Centre Laboratory. The electricity charges will vary from state to state depending on local rates per unit.

## CHART-II

\*PLAN OF A TUBERCULOSIS BACTERIOLOGICAL LABORATORY USED FOR THE PURPOSE OF CALCULATION OF COST



### Furniture

The furniture requirement has been worked out on the basis of experience gained at NTI laboratory and the area of the different sections of the laboratory (Chart 2). The cost of furniture in each facility and the rooms where each examination is carried out has been considered for calculating the depreciation value of the furniture required for each one of them. The depreciation value of the furniture in Bacteriologist's, Registration Clerk's and Store rooms has been apportioned to different facilities and examinations under the title 'common furniture'. Sectional furniture items are those exclusively used in each facility or the section where particular examination is carried out, e.g. furniture in washing and sterilisation, microscopy, culture rooms etc.

### Equipment & Supplies

The list of equipment and other articles required has been prepared according to the recommendations in WHO/TB/Tech. Guide/67.7 and based on the experience gained at NTI laboratory. For articles which are easily available in India and also for those not supplied by UNICEF, the local costs have been taken. For other articles received from UNICEF a conversion rate of Rs. 7.50/- per dollar has been adopted. In the case of certain items only part of the recommended quantity considered sufficient for carrying out the work has been included for calculation purpose. While estimating the

quantity of non-expendable and expendable articles required, sufficient allowance has to be made for wastage and breakage. In the case of slow moving glassware like McCartney bottles which will be held up for long periods in the incubator and cold room at least 3 times the actual requirement of a cycle has to be procured for smooth flow of work.

For finding out the depreciation value, the total cost of the equipment and the spare parts has to be divided by the number of years these are likely to be useful. Instead of charging separately for annual repairs a higher depreciation value can be charged to make calculations easier. It is better to have a smaller stock and charge a higher depreciation value than to have a larger stock and charge a smaller depreciation value, as the latter would mean more initial investment. The depreciation value for glassware has to be necessarily high as any crack or break will make them absolutely useless.

There are certain items of equipment which are not used exclusively in any facility or for any examination but used by all and the depreciation value of these has been apportioned among different facilities and examinations (according to the extent of utilisation) under the title 'common articles.' To this can be added the depreciation value of equipment and non-expendable items which are exclusively used in each facility or for each examination (sectional equipment). The cost of expendable articles

has been worked out on the basis of the annual consumption.

*Water Charges*

No expenditure under this head has been shown in the present calculation as water is generally supplied free to government institutions. But, wherever charges are levied this has to be shared by different sections according to the quantity used.

**Essential Facilities**

Those required are, (1) Cold room (2) Incubator room, (3) Gas room, (4) Washing and Sterilisation and (5) Media preparation. The annual expenditure on each of these has to be worked out first so that the total expenditure is shared by those utilising them.

(7) *Cold Room*

The cost of equipment and the installation charges paid by NTI have been adopted for calculation of expenditure. This will not vary much for a given size of the rooms irrespective of the work load in the laboratory. The cost of the furniture in these room has been worked out for the size of the racks that can be accommodated and for this also the price paid by NTI has been taken. The sum of the depreciation value of the equipment and furniture, part of the installation charges and

the share of expenditure on heads constitute the total expenditure for these two rooms. These amount to Rs. 2820/- for cold room and Rs. 1,220/- for incubator room. These have been apportioned to other essential facilities and examinations depending on the requirement of space for keeping cultures and other materials.

(2) *Gas Room*

The depreciation value of the gas plant and gas pipes and part of the installation charge and charge for laying gas pipes, electricity charges, share of rent and cost of petrol required for production of gas added together give the annual expenditure. The quantity of petrol required was taken as half the requirements of the NTI laboratory where 25,000 specimen were processed annually. The total expenditure on gas comes to Rs. 840/- and this has been distributed to other essential facilities and examinations depending on the number of burners used. The direct shares of expenditure on these 3 essential facilities viz., cold room, incubator room and gas room for other facilities and examinations are shown in Table 2.

(3) *Washing & Sterilisation*

The expenditure in this facility comes to Rs. 14,201- and this has been apportioned to media preparation and different examinations

TABLE 2

*Apportioning of expenditure (in rupees) under some essential facilities to other essential facilities and examinations*

Essential facilities and examinations	Gas plant	Incubation room	Cold room
1	2	3	4
Washing and Sterilization	168	—	—
Media Preparation	168	121	1903
Sputum Smear Examination	32	—	—
Primary culture— Concentration Method	220	732	282
Sensitivity Tests	189	305	476
Indentification Tests: Niacin	21	31	53
Catalase and Peroxidase	21	31	53
Room Temperature	21	-	53
Total	840	1220	2820

depending on the extent of utilization of this service ( Table. 3)

TABLE 3

*Apportioning of expenditure (in rupees) under washing & sterilisation to other essential facilities and various examinations*

Examination facility and examinations	Washing and sterilization
1	2
Media preparation	5680
Sputum smear examination	—
Primary culture: Concentration Method	5681
Sensitivity tests	2130
Identification tests: Niacin	237
Catalase and Peroxidase	237
Room temperature	237
<b>Total</b>	<b>14202</b>

#### (4) Media Preparation

Media are required for primary culture to all specimens and for sensitivity and identification tests in the case of positive specimens. The expenditure on media preparation will therefore depend upon number of specimens to be cultured and the number likely to become positive. While working out the requirement of media, besides wastage due to breakage and contamination, media required for sterility test also has to be included. The expenditure on Lowenstein Jensen medium is distributed among different examinations according to the quantity of media used. Similarly, expenditure on agar and broth has been distributed for the different examinations. These two media are used mainly for sterility tests which are essential for having a close check on the quality of sterilisation and also on technical procedures. Table 4 shows the details of apportioning the expenditure incurred on this item.

The third step in cost calculation is apportioning of expenditure under different heads and essential facilities to various examinations for which cost has to be found out. As explained earlier, the expenditure under heads has to be shared by the essential facilities and examinations, and the expenditure under facilities in turn has

TABLE 4

*Apportioning of expenditure under media preparation to various examination*

Examinations	Media Preparation
1	2
Sputum smear examination	—
Primary culture: Concentration Method	7027
Sensitivity tests	7019
Identification tests: Niacin	911
Catalase and Peroxidase	911
Room Temperature	911
<b>Total</b>	<b>16779</b>

to be shared by the various examinations. In the case of washing and sterilisation and media preparation, a part of the expenditure of other facilities has also been shared by them. The apportioning of expenditure is done on various bases such as work load, time involved in procedures, extent of utilisation of various facilities etc., and for this one's experience, knowledge of procedures involved etc., are the guiding factors. It is not possible to be too accurate in this.

The last step is to find out the cost of different examinations by totalling the direct share of expenditure of heads and essential facilities shown in tables 1 to 4. These are presented in a consolidated form in Table 5. Based on the number of specimens dealt with, the unit cost of different bacteriological examinations are worked out and presented in Table 6.

The important points to be remembered costing are:

- (1) No expenditure or depreciation value of any item should be omitted.
- (2) The cost of each and every item is interlinked with one another and so mistakes committed in one will have repercussions in others.
- (3) The calculation of cost for various items should be done as far as possible in th?

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TABLE 5

*Annual cost (in rupees) of different examinations for 12000 specimens*

Heads/and essential facilities	Sputum smear examns.	Primary cultures	Sen. test	Culture smear	Identification tests		
		Con. Method			Niacin	Cat. and perox.	Room temp.
1	2	3	4	5	6	7	8
Establishment	3698	5313	3758	—	501	501	250
Rent	1270	603	452	—	50	50	50
Electricity	33	100	100	—	11	11	11
Furniture:							
Common	18	19	14	2	2	2	2
Sectional	66	36	27	6	3	3	3
Equipment and supplies:							
Common	38	209	51	3	6	6	6
Sectional	1321	1939	83	291	113	78	31
Gas	32	220	189	—	21	21	21
Incubation room	—	732	305	—	31	31	—
Cold room	—	282	476	—	53	53	53
Washing and sterilization	—	5681	2130	—	237	237	237
Media preparation	—	7027	7019	—	911	911	911
Total	6476	22161	15356	302	1939	1904	1575

order in which the procedures are followed, so that the cost of certain general and basic components are worked out earlier and made available, for being included in the expenditure on any examination.

(4) Costing should be done strictly on commercial principles, though in the normal working of government institutions no depreciation is charged for anything. As and when equipment go out of order either they are repaired or replaced. If costing is done on this principle the first year's cost would be terribly high and in subsequent years there would be a precipitous fall.

*Cost of various examinations*

The cost of various examinations are presented in Table 6. It is evident that smear examination is cheaper than culture and its cost is less than one third of the cost of primary

culture. But, still considering the simplicity of the method it is not very cheap as expected generally.

The cost of sensitivity test is rather quite high and is almost 3 times the cost of primary culture and 10 times the cost of smear examination. The total cost of identification test is as much as cost of primary culture. These two tests viz., sensitivity and identification tests together make the cost of examination of a positive specimen too costly, i.e. about 4 times the cost of an examination of a negative specimen which will undergo only microscopic examination and primary culture.

One interesting fact that has come to light in the present calculation is that swab method of culture inspite of its simplicity and the need for practically no costly equipment is costing as much as the homogenisation and concentration

TABLE 6

*Unit cost (in rupees) of various bacteriological examinations in a State Tuberculosis Centre Laboratory with annual work load of 12,000 specimens of which 25% are positives*

Examinations	Cost
1	2
Smear Examination	0.54
Primary Culture	
(a) Swab Method	1.86
(b) Concentration Method	1.85
*Sensitivity tests	5.22
Identification tests: Niacin	0.65
Catalase and peroxidase	0.63
Growth at Room temperature	0.53
(Total of 3 Identification tests)	(1.81)
Total for all examinations with primary culture by:	
Swab Method	9.43
Concentration Method	9.42

\*Includes cost of examination of culture smears and H37 RV control. The sensitivity test for H37 RV costs Rs. 7.78 as the number of drug slopes used is more.

method of culture for which costly equipment like centrifuge is required.

### Cost of setting up of a laboratory

In the present paper, the cost of primary culture for the two methods generally followed viz., swab method and concentration method has been worked out so that the total expenditure for all examinations whether recurring or nonrecurring can be found out separately with the primary culture by any one of the methods. This has been done to enable smaller laboratories which cannot afford to follow both the methods of culture to choose the method they would like to adopt.

The initial expenditure for setting up a laboratory will always be higher than its recurring expenditure because of spending huge

amounts in purchasing equipment and furniture. The non-recurring and recurring expenditures of the State Centre Laboratory are presented in Table. 7. It will be seen that non-recurring expenditure (initial expenditure) is about twice the recurring expenditure. The first year expenditure of the laboratory can be found out by adding up the non-recurring and recurring expenditure and this comes to about Rs. 1,50,000/- i.e. nearly 3 times the recurring expenditure. It is also seen that the salary of the staff alone claims nearly 50% of the recurring expenditure of about Rs. 50,000/-

The depreciation value of the equipment and furniture comes to about Rs. 12,000/- and expenditure on maintenance of equipment and furniture per annum can be taken as 10% of the amount.

### Discussion

An attempt to work out the cost for establishing a Tuberculosis Bacteriological Laboratory and the cost of various bacteriological examinations generally undertaken in such a laboratory has been made. The calculations presented here will be helpful for health administrators for planning and execution since these will give an idea of what it would cost to establish a laboratory and how much various examinations would cost.

A pertinent point to be investigated while working out the cost of any laboratory service is, whether the staff appointed and equipment purchased are fully utilised so that the organisation gets the maximum returns for the investments made. For this, three factors are to be considered viz., the staff potential, the equipment potential, and the investment made in both. If the cost of equipment is not high in relation to the salary of the staff, potentialities of the staff should be exploited to the maximum. On the other hand if the expenditure on equipment is rather high in relation to that of the staff, full potentialities of the equipment have to be exploited. Even if there is not enough use for the equipment in a particular organisation, its services can be offered to other institutions on hire.

No attempt seems to have been made so far to work out the cost of various examinations in a Tuberculosis Bacteriological Laboratory. However, costing has assumed importance with the launching of Tuberculosis Control Programmes in the developing countries. Under the programme, the diagnosis is mainly by sputum smear examination and only for assessment purpose at State level culture examination is

TABLE 7

*Non-recurring expenditure of State Centre Laboratory (in rupees)*

Sl. No.	Items	Non recurring expenditure			Recurring expenditure		
		Furniture	Equipment	Total	Expendable and others	Depreciation value & maintenance expenditure	Total
1	2	3	4	5	6	7	8
1.	Staff	—	—	—	24312	—	24312
2.	Rent	—	—	—	7200	—	7200
3.	Electricity (cost of bulbs, tube lamps, fittings and current charges)	—	508	508	1266	64	1330
4.	Gas plant (cost of gas plant, gas pipes, installation charges, petrol)	—	4125	4125	280	275	555
5.	Cold room (cost of plant installation charges etc.)	732	21461	22193	—	1677	1677
6.	Incubator room (cost of plant, installation charges etc.)	512	10522	11034	92	611	703
7.	Common articles	—	4794	4794	249	587	836
8.	Common furniture	1929	—	1929	—	96	96
9.	Distilled water* equipment	—	1434	1434	—	—	—
10.	Washing & sterilisation	2689	18133	20822	845	2365	3210
11.	Media preparation	824	13336	14160	1734	3305	5039
12.	Microscopy (sputum smear)	1442	3558	5000	1033	379	1417
13.	Primary culture: Swab Method	713	8820	9533	1022	1170	2192
	Concentration Method	713	12455	13168	443-	1531	1974
14.	Sensitivity tests	713	4913	5626	80	883	963
	Culture smear	—	2897	2897	112	155	267
15.	Identification tests;						
	(a) Niacin test	—	34	34	78	3	81
	(b) Catalase & peroxidase test	—	—	—	48	—	48
	(c) Room temperature	—	—	—	—	—	—
	(a) Swab method	9554	94535	104089	38356	11570**	49926
	Total for all (b) Concentration method with primary culture by	9554	98170	107724	37777	11932**	49709

\*The recurring expenditure on distilled water equipment is omitted as it has been shared by other items following as cost of distilled water under expendables.

\*\* The annual maintenance expenditure may be about 10% of depreciation value.

advised. It is often questioned as to why diagnosis by culture should not be undertaken at district level as there will be higher yield of cases, culture being more sensitive than microscopy. Rao et al 1971 have shown that among culture positive patients who could produce sputum, 85% of the cases can be diagnosed by microscopy alone. Therefore, the 15% addition by culture has to be judged against the cost of culture examination. The average cost of examination of a specimen by culture comes to Rs. 4/- and out of this only about one-eighth is spent on smear examination and the rest is spent on culture, sensitivity tests etc. Another point that cannot be ignored is the cost of setting up of a tuberculosis bacteriological laboratory. In a country like India with limited resources and with more than 340 districts, to establish and run culture laboratories in every district would mean even on a conservative estimate spending more than 50 million rupees. Once the laboratory is established in a district, arrangements for getting specimens from various parts of the districts for culture examination have to be made. This will further boost up the cost of culture examination.

The cost of smear examination is less than that of culture examination, but still 60% of the expenditure on smear examination is on staff. Because of this one could expect the smear examination to be costlier in District Centres than in peripheral health institutions, where no specialised staff are appointed for direct microscopy.

Under the TB programme culture examinations are recommended only for assessment, studies on prevalence of drug resistance etc. However, to help the staff to gain sufficient experience of performing various tests, and to attain and maintain an adequate standard for undertaking such work in future, a certain amount of culture work including performance of various tests have to be carried out as a routine. Therefore, it becomes obvious that the laboratory cannot be left idle till some studies are undertaken, or just for the sake of engaging the staff the Centre cannot undertake assessment work or some studies continuously.

Though the cost calculated for any item may vary from place to place depending on the local market rates, the cost worked out once can be valid only till such time the expenditure incurred remains steady. The cost of various examinations presented in this paper will hold good only for the particular set of situations described and if situations are different, cost

will vary. The staff strength, their pay scales, the number of specimens to be cultured, the number likely to become positive and undergo sensitivity and identification tests, and the technical procedures laid down will influence the cost to a very great extent and any alteration in any one of these factors will give rise to different rates for various examinations. Therefore, it is not possible to work out common cost for various examinations which will hold good for all the institutions.

### Summary

The cost of establishing and running a Tuberculosis Bacteriological Laboratory in the State Tuberculosis Centres under the National Tuberculosis Programme, and the cost of various examinations to be undertaken in such a laboratory have been presented. Only a brief description of this methodology of the calculation has been given with set of tables showing the expenditures incurred for the various items. Certain interesting findings have been highlighted.

The place of smear and culture examination under the programme, the implications of establishing a culture laboratory, the limitations of cost worked out have been discussed.

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### REFERENCE

- (1) Rao K.P. et al (1971) *Ind. J. Tub.* Vol. XVIII/1 PP. 10 to 21.

(1) The cost of various examinations was worked out on the basis of the prices of equipment and materials prevailing in 1967 or earlier, and present costs for various examinations therefore will be appreciably higher than what has been stated. However, the relative cost may remain the same.

(2) The full details of the calculation and the technical procedures adopted in the laboratory for this purpose can be had on request from the National Tuberculosis Institute, Bangalore. 3, India.

## A RETROSPECTIVE ANALYSIS OF YIELD OF CASES BY TWO METHODS OF SPUTUM COLLECTION IN SUSPECTS OF PULMONARY TUBERCULOSIS AMONG SYMPTOMATICS ATTENDING A TUBERCULOSIS CENTRE

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The two methods for establishing bacteriological laboratory diagnosis of pulmonary tuberculosis are detection of acid-alcohol-fast bacilli in sputum smears and mycobacterium tuberculosis in the cultures. Direct microscopy has been advocated to be an effective case finding method by several workers (1, 2, 3, 4) while some others have found it not so convincing (5, 6).

In developing countries National Tuberculosis Programmes are being evolved on the basis of case detection by direct microscopy. The results of microscopy would much depend on various factors including type of facility, and type and number of specimens collected (7, 8, 9, 10, 11, 12).

From the point of view of prevailing conditions of paucity of staff, training, supervision, non-provision of culture facilities, under-diagnosis (12) in the national tuberculosis control programme, coupled with other operational factors like unwillingness on the part of some persons to wait for the result of microscopy on the day of examination, it becomes rather necessary to find out the relative utility of Direct Smear Examination of a 'collection' specimen of sputum for proper diagnosis of Pulmonary Tuberculosis.

### Material

The data refer to diagnostic sputum examination of 186 out-patients with a provisional diagnosis of pulmonary tuberculosis made at T.B. Demonstration and Training Centre, Agra, where they had presented with symptoms. For the purpose of the present comparison it was stipulated that the results should be available of examination in the centre, by direct smear and by culture, of two specimens (one smear from each specimen) from each patient, namely one unsupervised immediate ('spot') and one unsupervised early morning ("collection") specimen. Only those patients who had a positive finding in at least one of the two specimens, i.e. with acid-alcohol-fast bacilli morphologically resembling myco. tuberculosis in a direct smear or with colonies typical of myco. tuberculosis on culture were included in the analysis. Out of 186 suspects 160 were considered to have bacteriologically confirmed

pulmonary tuberculosis. Most of the patients did not give specific history of their previous anti-tubercular treatment. As far as could be ascertained from repeated questioning, 99 patients had 20 injections or less or none at all, and 61 patients had more than 20 injections before coming to this centre. Sixteen patients were found resistant; 4 amongst those who had 20 injections or less and 12 amongst those who had more than 20 injections.

Out of 160 patients, 100 (62.5 percent) were male and 60 (37.5 percent) were female. The age ranged from 10 to 70 years, 85 percent of the males and 75 percent of the females being between 20 and 50 years of age. All these patients had radiographic appearances compatible with the diagnosis of pulmonary tuberculosis. All the patients had at least moderately advanced disease.

### Method of sputum collection

(a) A 'spot' specimen was one produced in the premises of this Centre. The person was asked to cough and expectorate into a wide mouth, Borosil glass bottle. The specimen was the material, either sputum or saliva which the person produced upon demand within a matter of a few minutes.

(b) A 'collection' specimen was one produced in the early morning hours within a matter of few minutes, before cleaning the mouth.

Both the sputum specimens were unsupervised collections. The specimens for diagnostic purposes were almost invariably collected within a week. As the results of 70 mm Photo-fluorogram of the chest were available on the day of the first visit of the person to the clinic, a 'spot' specimen of the sputum was obtained between 12.30 p.m. and 2.30 p.m. on the same day; in addition a sterile bottle was given for his 'collection' specimen which was obtained the next day between 8.30 a.m. and 11.30 a.m., according to the current practice in this centre. All the persons also accordingly were given a Tuberculin test with 1 TU RTXXIII in 0.1 ml phosphate buffer containing 0.005 percent tween 80, the results of which were read after 48 hours.

*Bacteriological procedures*

In the laboratory the 'spot' and the 'collection' specimens were treated in an identical manner but the possibility that the staff were aware of the type of specimen when processing them could not be denied (a situation commonly seen in most of the Centres).

Each specimen was examined by the following techniques.

(a) Microscopy of Direct smear : Ziehl-Neelsen technique.

A new glass slide was used for the preparation of each smear. Purulent material, if present, was selected from the specimen, failing which mucoid material or the deposit in the saliva was used. The smear was fixed, stained by standard Ziehl-Neelsen technique and examined the same day. Positive smears were graded as 1-plus, 2-plus or 3-plus according to the average number of bacilli seen per field.

1-plus:— less than one bacillus per field. 2-plus:— one to 10 bacilli per field. 3-plus:— More than 10 bacilli per field.

(b) Culture :— Lowenstein-Jensen medium was used as described by Hoist and others (1959).

Cultures were examined weekly for eight weeks and were reported as negative if no growth was present by the end of that time. Growth typical of myco. tuberculosis was graded as under :—

- (i) Actual Number:— If the number of colonies was less than 20.
- (ii) 1-plus :- If there were 20 to 100 colonies.
- (iii) 2-plus :— If there were innumerable discrete colonies.
- (iv) 3-plus :— If the growth was confluent.

Cultures that yielded tubercle bacilli were identified by sub-culturing and observing growth at room temperature, production of pigment in the dark and after exposure to day light, rate of growth at 25°C and 42°C, P.N.B. test (500 (Jig/ml) and Niacin production.

**Results**

Among 186 suspects of pulmonary tuber-

culosis, 157 had positive cultures for mycobacterium tuberculosis, 5 had cultures showing growth of unclassified myco bacteria, 23 had negative cultures and in one culture was contaminated.

On the basis of direct smear and culture results 160 persons were considered to have bacteriologically confirmed pulmonary tuberculosis.

(a) *Comparison of direct smear examination of one 'spot' and one 'collection' specimen of sputum.*

The results of direct smear and culture examination of the two diagnostic specimens (one 'spot' and one 'collection') from 160 persons considered to have bacteriologically confirmed pulmonary tuberculosis are shown in table I. On direct smear examination 83(51.8 percent) of the 'spot' specimens were positive as compared to 136 (85 percent) of the 'collection' specimens. The difference in the positivity rate is statistically significant ( $P < 0.01$ ), a finding similar to that observed by Andrews et al(1).

Of 83 smear positive 'spot' specimens, 4 (4.8 percent) were graded as 3-plus whereas, of the 136 smear positive 'collection' specimens 29 (21.3 percent) were graded as 3-plus. Taking into account this difference in the grading of positivity, the contrast between the results from the two types of specimen is significant

Of the 83 smear positive 'spot' specimens 56 (67.5 percent) were graded as 1-plus, whereas of the 136 smear-positive 'collection' specimens 52 (38.2 percent) were graded as 1-plus. This difference in the grading of positivity from the two types of specimen is statistically significant ( $P < 0.01$ ).

None of the 83 positive results revealed by direct smear examination of the 'spot' specimen was missed by direct smear examination of the 'collection' specimen. In addition the 'collection' specimen detected 53 (32 percent) more cases as compared to the 'spot' specimen.

For the purpose of grading of positivity, the 'collection' specimen was better than the 'spot' specimen (Table II), The proportion of specimens with high bacillary content (graded 3-plus) was significantly more in the 'collection' specimen ( $P < 0.01$ ) and hence lesser chances of under-diagnosis and more chances of case detection; a similar finding has also been reported by Andrews et al (1).

**TABLE I**  
*Results of direct smear and culture examination of 'spot' and 'collection' specimens of sputum in 160 patients considered to have bacteriologically confirmed pulmonary tuberculosis*

No. of specimens per patient	Type and combination of the specimens	Total number of patients	Direct smear				Culture									
			Positive			Negative	Positive			Negative						
			3-plus	2-plus	1-plus		Total	3-plus	2-plus		1-plus	Total				
1	'spot'	160	4	23	56	83	51.8%	77	48.2%	11	45	91	147	91.8%	12	7.5%
1	'collection'	160	29	55	52	136	85 %	24	15 %	62	47	48	157	98.1%	2	1.2%
2	one 'spot' and one 'collection'	160	31	59	46	136	85 %	24	15 %	62	47	48	157	98.1%	2	1.2%

Note : Where there is more than one specimen per patient, 'positive' means positive on any one of the specimens and 'Negative' means negative on all the specimens.

(ii) Culture results not available for one patient due to culture contamination.

TABLE II Comparison of microscopy results for 'spot' and 'collection' specimens

	'Spot' specimen					
	Positive				Negative	Total
	+++	++	+	Total		
'Collection' specimen						
I Positive +++	2	10	8	20	9	29
++	1	8	25	34	21	55
+	1	5	23	29	23	52
Total	4	23	56	83	53	136
II Negative	—	—	—	—	24	24
Total	4	23	56	83	77	160

## (b) Comparison of culture results of one 'spot' and one 'collection' specimen

On culture examination, 147 (91.8 percent) of the 'spot' specimens were positive as compared with 157 (98.8 percent) of the 'collection' specimens. Of 147 culture positive 'spot' specimens 11 (7.4 percent) were graded as 1-plus whereas of the 157 culture positive 'collection' specimens 62 (39.4 percent) were

graded as 3-plus and 48 (30.5 percent) were graded as 1-plus (Table 1). These differences in the grading of positivity of the two types of specimens were statistically significant at 1 percent level, probably due to qualitative inferiority of the 'spot' specimens and variation due to other factors; hence for the purpose of grading of positivity of cultures and case detection 'collection' specimen was better than the 'spot' specimen. (Tables I, III).

TABLE III Comparison of culture results for 'spot' and 'collection' specimens

	'Spot' specimen					
	Positive				Negative	Total
	+++	++	+	Total		
'Collection' specimen						
I Positive +++	11	27	20	58	4	62
++	—	18	27	45	2	47
+	—	—	44	44	4	48
Total	11	45	91	147	10	157
II Negative	—	—	—	—	2	2
Total	11	45	91	147	12	159

(c) *Comparison of a single 'collection' specimen with two specimens.*

Direct smear examination of a single 'collection' specimen as well as direct smear examination of two specimens yielded 85 percent of the 160 positive results (table 1) and thus examination of single 'collection' specimen was found to be as effective as examination of two specimens (one 'spot' and one 'collection'). On culture examination, the 'collection' specimen yielded 98.1 percent of the positive results. Andrews et al (1) showed that direct smear examination of two specimens yielded 83.3 percent by direct smear examination of a single 'collection' specimen and the difference was not significant statistically. Chandrashekhar et al (9) reported that more than 80 percent of the smear positives (confirmed by culture) could be found from examination of one specimen only.

### Discussion

For the purpose of diagnosis in persons presenting with symptoms suggestion of Pulmonary Tuberculosis and having moderate or extensive radiographic disease, a 'spot' specimen can be valuable. It has the advantage of requiring only one attendance and therefore minimum of cooperation from the patient. Production of a 'spot' specimen may be more acceptable to the persons than a 'collection' specimen and further advantage is that a positive result may in many persons be obtained on direct smear examination without the delay necessitated by waiting for a culture result. In addition, this type of specimen has without question been obtained from the person concerned and not from some other person and thus if supervision can be introduced there are no chances of collecting a wrong specimen.

A 'collection' specimen which requires two attendances gives significantly higher case yield and for the purpose of grading of positivity it is significantly superior to a 'spot' specimen. In the present series examination of a single 'collection' specimen was found to be as effective as examination of two (a 'spot' and a 'collection') specimens. It was also revealed that culture examination as a routine is not essential since 98 percent of the direct smear positive 'collection' specimens were confirmed by culture.

These findings indicate that in situations where a patient can attend a facility on two occasions, a 'collection' specimen should be preferred in case only one specimen is to be

examined and that culture as a routine examination may not be necessary.

### Summary

A comparison is presented between the results of examining a 'spot' and a 'collection' specimen of sputum (both unsupervised) from 160 patients with bacteriologically confirmed Pulmonary Tuberculosis, using the results of direct smear examination by Ziehl Neelsen technique and of culture on Lowenstein Jensen medium.

Direct smear examination of one 'collection' specimen yielded 85 percent of positive results and 98 percent of these were confirmed by culture. Significantly higher proportion of cases known to have positive sputum were detected by a 'collection' specimen as compared to a 'spot' specimen. Moreover, grading of positivity showed an advantage to the 'collection' specimen over the 'spot' specimen.

While inferior to a 'collection' specimen, a 'spot' specimen can yield useful information, particularly if culture facilities are available. The presence of Myco. tuberculosis was demonstrated by direct smear examination in 51.8 percent and by culture in 91.8 percent of patients known to have a positive sputum.

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## MANAGEMENT OF SPONTANEOUS BRONCHO-PLEURAL FISTULA IN CHILDREN (STUDY OF 30 CASES)

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Langston (1966) has recently called attention to what he considers significant "blind spots" in the current management of thoracic empyema in children complicated with broncho-pleural fistula: (i) undue persistence in closed drainage procedures, (ii) over-enthusiasm for decortication as a method of mechanical cleansing of the pleural cavity. The report analyses the experience and problem posed in cases of broncho-pleural fistula complicating empyema in children. Bryant (1968) mentioned the delay in referring the case of empyema in paediatric group, and discussed the management of empyema with broncho-pleural fistula in children. Tom. M. Johnson (1973) discussed the problems in treating cases of broncho-pleural fistula. He observed that broncho-pleural fistula is seen today (U.S.A.) in patients with old, healed tuberculosis especially in those with previous pneumothorax and no chemotherapy. The diagnosis is considered in those patients on the basis of an increasing but variable amount of sputum production, air in the pleural space and changing air fluid level. Once the diagnosis is made, treatment with anti-tuberculosis chemotherapy combined with temporary drainage procedure to the exterior should be undertaken. After control of the active disease, the patient should have definite surgical treatment consisting of procedures designated to fill the pleural space such as staged thoiacoplasty and decortication. It is advisable to continue combination chemotherapy for a 2 year period and to administer isoniozid for the patient's lifetime.

### Material and Methods

For the purpose of this study only those patients with frank broncho-pleural fistula presenting with tension pneumothorax or hydro-pneumothorax were included in the analysis. 30 children below the age of 12 years were admitted under the care of thoracic unit of the S.N. Medical College, Agra. These children were seriously ill at the time of admission & either came directly or were referred by the general practitioners or paediatric department of Medical College, Agra. Detailed history including the clinical findings were carefully recorded. After routine investigation of blood, urine, stool, all of them had screening and X-ray of the chest to locate the site and extent of fluid, shift of mediastinum and to see

whether the opposite lung was healthy or not. Oblique and lateral views were taken whenever indicated. Bronchography and Sinography were done in all cases to confirm the diagnosis and to localise the size and site of fistula. Pleural fluid was aspirated in all children and the pus was sent for culture and sensitivity testing for pyogenic organisms and acid fast bacilli.

TABLE I  
*Age distribution*

Age Group	No. of cases	Percentage
0-2	4	13.3
3-4	5	16.7
5-6	7	23.3
7-8	8	26.7
9-10	4	13.3
11-12	2	6.7

The cases were evenly distributed in different age groups. Fifty percent of the children were in the 5-8 years group.

There were 22 males and 8 females with ratio 2.7 : 1. The disease was more common on the right side-18 cases (60 percent)-than on the left side-12 cases (40 percent).

Duration of illness varied from a few weeks to a few months. It was 1-2 months in about one-third of the cases.

TABLE II  
*Duration of disease*

Duration of months	No. of cases	Percentage
0-1/2 month	6	20.0
1-2 months	9	30.0
3-4 months	7	23.3
5-6 months	5	16.7
6-8 months	3	10.0

All the 30 children were acutely ill; they were dyspnoic, orthopnoic, toxæmic, with high pyrexia, emaciation, loss of weight, cough and expectoration. 8 cases (26.7%) who had large fistula gave history of coughing of the pus which used to increase with a choking sensation in some postures. All of them had clinical and radiological picture of hydropneumothorax with shift of mediastinum to the opposite side. Empyema necessitatus was present in 4 cases (13.3%), liver was palpable in 18 cases (60%) and signs of congestive heart failure with general anasarca was present in 6 cases (20%).

Twenty five of the 30 cases had symptoms suggestive of tuberculosis viz. cough fever, loss of weight. Out of these 25 children 10 (33.3 %) had a family history of tuberculosis while 15 patients (50%) were taking the treatment. The diagnosis in these cases were made on previous X-ray of the lung which showed cavitation or infiltration in the lung fields.

Sixteen children (64 %) out of 25 had manifestation of tuberculosis in other part of body in the form of tuberculosis of lymph nodes in the neck confirmed on biopsy in 12 cases and contralateral pulmonary disease in 4 cases (16%). Acid fast bacilli could only be isolated in 8 cases either from the sputum (3 cases) or pus (5 cases).

The remaining five cases (16.7%) out of 30 cases gave acute history of febrile illness in a healthy child and were admitted in paediatric department with a diagnosis of staphylococcal abscess. The staphylococci were isolated from the sputum of the first three cases, while mixed organisms E-coli, Staphylococcal and proteus were isolated from the pus in the other 2 cases. First three cases of staphylococcal pneumonia developed empyema with fistula while under treatment for pneumonia which manifested with rising temperature, increase in toxæmia with dyspnoea and pain in the chest. The skiagram showed signs of hydro-pneumothorax. Various aetiological types of empyema seen in the present study are shown in Table III.

All the patients were given effective medical treatment to build up general condition and appropriate antibiotic to sterilise the pleural cavity. In tuberculous cases three drugs were used at a time. Streptomycin, Isonex, Unipyrazinamide were given in 17 cases and the other 8 who were economically better off were put on Isonex, Ethambutol and Unipyrazinamide. The three drug treatment was strictly followed for 18 months to 24 months till the lesion healed completely and the patient was completely asymptomatic for period of not

TABLE III  
*Aetiological types*

Aetiological types	No. of cases	Percentage
1. Tubercular: 25 cases		
A. AFB isolated from sputum or pus.	8	26.7
B. T.B. in other parts of the body.		
1. Gland	12	40.0
2. Opposite lung	4	13.3
C. Past history of tuberculosis	1	3.3
2. Staphylococca		
(a) Pneumonia	3	10.0
(b) Pneumonia with Broncho-pleural fistula.	2	6.7

less than six months. All of them in addition to the medical treatment required surgical procedures, depending on the size of the fistula and the condition of the underlying lung. Various surgical procedures used in the present series are shown in Table IV.

TABLE IV  
*Surgical procedures*

Surgical procedure	No. of cases	Percentage
Intercostal drainage under water-seal (closed)	2	6.7
Open drainage with intercostal tube	18	60.0
Decortication with closure of fistula	4	13.3
Closure of fistula	2	6.7
Thoracoplasty	4	13.3

### Discussion

Repeated aspiration was attempted in all cases but it completely failed to expand the

lung in the presence of broncho-pleural fistula. All the 30 cases had intercostal drainage under water seal, huge amount of pus drained out with air leak. The fistula with a small bronchial leak closed spontaneously and the lung expanded to obliterate the empyema cavity in only 2 cases. The pus gradually decreased in amount and the tube was eventually removed. These were the cases following rupture of staphylococcal abscess. The remaining 28 cases had large bronchial leak and in these cases the lung failed to expand. The 18 cases (60%) had open drainage. In this group were included three types of patients, those who had tuberculosis lesions in glands or the opposite lung, secondly those who refused operation, thirdly those children who were considered poor subjects for surgery. In these children 1/2" of rib at the bottom of empyema cavity was resected, large size tube was put in and left for open drainage into the dressing. In all these patients, empyema cavity was slow to obliterate. Gradually the discharge decreased and the space reduction was checked by repeated sinography. The tube was removed when the quantity of pus discharge was very small (less than 1 ml) and only a track was visible in the X-ray with no empyema cavity. In these cases our impression was that the bronchial leak gradually decreased due to healing of fistula and the under-lying lung partially expanded to obliterate the empyema space. The obliteration of empyema space was further facilitated by gradual shift of the mediastinum and hypertrophy of the opposite lung which gradually took over the function of trapped lung. The pliable chest wall of children hastens the healing of empyema cavity from the periphery. The time taken in these cases was much longer (6-12 months with average 8 months).

10 cases (33 %) out of 30 cases required major surgical procedures. Four cases were treated by decortication with closure of fistula. They had thickened visceral and parietal pleura with bronchial leak, but the collapsed lung was healthy and disease process was localised. The lung expanded and obliterated the pleural space. Two of the four cases were of resolved staphylococcal empyema and the remaining two cases had a cavity at the apex which ruptured in the pleural cavity leading to broncho-pleural fistula with empyema.

The closure of fistula following rupture of tuberculosis focus was carried out in two cases only. These patients had received chemotherapy tuberculosis and were good subjects for surgery. The history was short and pleural infection was fairly recent with no secondary focus in the opposite lung. Following thoracotomy, the bronchial fistula was stitched with atraumatic silk and the pleural cavity was drained under water seal. One case remained well while in the other, stitches cut through the bronchial stump leading to recurrence of fistula, later on kept on open drain which recovered slowly.

4 cases (13.3%) of bronchial fistula with large air leak and diseased, collapsed lung were kept on chemotherapy and open drainage. Their response was poor as the diseased lung was collapsed and fibrosed with thickened visceral pleura. These cases had two stage total thoracoplasty from 2nd to 8th ribs to obliterate the empyema cavity. One case died of acute dilatation of the stomach which is one of the rare complications following thoracic surgery, and the other three did well.

#### Summary

Management of 30 cases of spontaneous broncho-pleural fistula is discussed. 25 cases developed following rupture of tubercular focus of lung while 5 developed following rupture of staphylococcal pneumonia of lung. Intercostal drain was life-saving procedure in these acutely ill children. Morbidity was high but responded to good chemotherapy and open drain of the empyema cavity in (60%) cases while 10 cases (33.3%) required major surgical procedures with high morbidity and small mortality. Only 2 cases (6.7%) with small bronchiolar leak responded to under water seal drain.

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## ELECTROPHORETIC PATTERN OF SERUM PROTEINS IN CHILDHOOD TUBERCULOSIS

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### Introduction

Ever since Robert Koch identified the Mycobacterium tuberculosis, an increasing number of workers have interested themselves in the study of its numerous exciting aspects. Though last few decades have seen remarkable improvements in our diagnostic and therapeutic armamentaria, attempts are still in progress for the establishment of more reliable criteria for assessing the progress of the disease after diagnosis and initiation of therapy. Amass of literature has accumulated on the value of clinical assessment, serial radiological examination, E.S.R. determination and repeated bacteriological examination for A.F.B. but inadequacies of these methods have prompted sojourn of scientific curiosity into more recent biochemical techniques.

Seibert and Nelson<sup>1</sup> first demonstrated the rise of gamma globulins in tuberculosis. A critical review of the literature reflects that the results obtained by different workers have not been very consistent.

In this paper we have presented results of electrophoretic pattern of serum proteins in various types of childhood tuberculosis in India. 35 cases with a follow up study are presented.

### Material and Methods

Thirty five children between the age groups of 1-15 years suffering from tuberculosis have been studied both in the inpatient and outpatient departments. Total duration of the study has been 1 year.

Only fresh and untreated cases were included in the study. Any condition which could account for disturbances in serum proteins was not included in the study even though associated tuberculosis was present.

All the cases were initially evaluated with a detailed history and clinical examination.

The following investigations were undertaken :-

1. Mantoux test using 1 : 100 old tuberculin.
2. A skiagram of chest—initially and repeated periodically for assessment of progress.

3. Hematological investigations by standard techniques.
4. Erythrocyte sedimentation rate by Wintrobe<sup>2</sup> method corrected for packed cell volume as described by Wintrobe and Landsberg.
5. Staining of smears from exudates for A.F.B. by Z.N. method.
6. Estimation of total serum proteins by Biuret method.
7. Estimation of differential serum proteins by paper electrophoresis by 'EEL' paper electrophoresis apparatus. Barbitone buffer pH 8.6 with a strength of 0.1 molarity was used as buffer.
8. Bacteriological investigation for A.F.B. by culture techniques.
9. Histopathological examination of biopsy material wherever necessary.

After the initial clinical examination and investigation all the cases were put on a predetermined treatment schedule. The cases were then followed up at monthly intervals clinically, radiologically and by serial determination of the serum protein fractions and E.S.R.

Twenty five normal children served as controls. These were drawn from Well Baby Clinic and Children's Ward.

### Observations

Thirty five children were included in the study and there were 19 female and 16 male children (Table I).

TABLE I

<i>Total no. of cases</i>	—	—	35
Female children	—	—	19
Male children	—	—	16
<i>Age Groups</i>			
Below 5 years	—	—	19
5—10 years	—	—	6
Above 10 years	—	—	10

*Age Groups* :- Nineteen children were below 5 years of age and six were between 5-10 years and ten were above the age of 10 years (Table 1).

*Types of cases* : There were seven cases of primary tuberculosis, seven cases of progressive primary tuberculosis, 8 cases of chronic pulmonary tuberculosis, 9 cases of generalised tuberculosis and 4 cases of lymphnode tuberculosis (Table 2).

TABLE 2  
*Type of cases*

Primary tuberculosis	—	—	7
Progressive primary tuberculosis	—	—	7
Chronic pulmonary tuberculosis	—	—	8
Generalised tuberculosis	—	—	9
Lymphnode tuberculosis	—	—	4
Normals —	—	—	25

*Diagnostic criteria (Table 3)*

Acid fast bacilli were isolated in 18 (51.4%) cases. Histological evidence of tuberculosis was present in 6 (17.5%) of cases. Positive Mantoux test, radiological evidence of tuberculosis and positive history of contact was present in 9 (25.71 %) cases. Positive Mantoux and radiological evidence was present in 1 case.

The normal ranges, means and standard errors of total protein and various protein fractions in controls are presented (Table 4).

*Changes in serum protein fractions in patients:- (Table 5)*

*Primary Tuberculosis* : There was no significant change in the total protein and serum albumin levels. Similarly there was no significant alteration in alpha<sub>1</sub>, alpha<sub>2</sub> and beta globulins. All the seven cases showed slight increase in gamma globulins. The range was 19.02 to 26.64 % with a mean of 20.44 % as against normal mean value of 16.51 %. On serial determination four of these cases started showing a downward trend after 3 months of treatment and had reached normalcy at the end of six months. In 3 cases, it showed downward trend only but did not touch the normal levels at the end of six months.

*Progressive Primary Tuberculosis*: In five of the seven cases the serum albumin level was at the lower limit of normal range, and in other two cases there was slight and moderate decrease. The mean value of 47.12 % for this group is definitely lower than the mean normal value of 59.40%. Four of the seven cases showed an increase in the alpha<sub>2</sub> fraction. The mean value for this group was 12.85% as against the mean normal values of 10.97%. All the seven cases showed an increase in the gamma globulin values. The range was 23.17% to 26.60% with a mean value of 24.79% (normal mean 16.51 %). On serial determination the albumin levels returned to normal levels in all cases. The alpha<sub>2</sub> fraction had returned to normal levels by

TABLE 3

*Diagnostic criteria, number and percentage of selected cases*

Diagnostic criterion	No. of cases	% of total
A.F.B. Isolated	18	51.44*
Histological evidence of tuberculosis	6	17.15*
Positive Mantoux, radiological evidence of tuberculosis, positive history of contact	9	25.71
Positive Mantoux, radiological evidence	1	2.85
Radiological evidence, positive history of contact	1	2.85

\*Corrected

TABLE 4

*Normal ranges, median and mean with standard error of protein fractions in 25 controls*

Protein fractions	Percentage range	Percentage median	Percentage mean	Percentage error
*Total Protejn	6.0— 7.2	6.7	6.3	—
Albumin	54.72—64.33	59.36	59.40	±0.545
Alpha <sub>1</sub>	2.72— 4.93	4.16	3.92	±0.137
Alpha <sub>2</sub>	8.98—12.91	11.56	10.97	±0.367
Beta	6.93—12.43	9.69	9.09	±0.348
Gamma	12.59—18.55	16.85	16.51	±0.398

\*Gms. Percent

the end of six months in three of the cases. However, gamma globulins did not completely return to normal levels even at the end of six months though a downward trend was observed.

*Chronic Pulmonary Tuberculosis* : Two cases showed a slight reduction in total proteins. All the eight cases showed a decrease in albumin fraction. The values range from 32.82% to 41.46 % with a mean of 38.09 % as against a normal mean of 58.40%. Alpl<sup>^</sup> and beta fractions were increased in only 2 and 3 cases respectively. alpha<sub>2</sub> was increased in all the 8 cases of this group. The values ranged between 14.35% and 20.45% with a mean of 17.26% (normal 10.97%). All the eight cases showed a marked increase in gamma globulin fraction. The values ranged from 24.86 to 34.17% with a mean of 28.88%. On serial determination at the end of 6 months, hypoalbuminemia persisted and alpha<sub>a</sub> showed a gradual fall. The gamma globulin fall was much slower.

*Abdominal Tuberculosis*: There were no changes in total protein values. Serum albumin showed a decrease in all the four cases the range being from 36.72% to 43.27% with a mean of 40.25%. No alteration was determined in alpha<sup>!</sup> and beta fractions. The alpha<sub>2</sub> showed an increase in all the four cases the range being 17.33% to 19.17% with a mean of 18.28%. All the four cases showed a marked increase in gamma globulin values the range being from 24.59 to 29.63% with a mean of 24.70%. On serial determination a fall in alpha<sub>a</sub> and increase in albumin values was significant. The gamma

globulins maintained their high levels throughout.

*Protected Hematogenous tuberculosis* : There was a moderate decrease in the serum albumin initially which returned to normal at the end of six months. The marked increase in gamma globulins and the moderate increase in alpha<sub>2</sub> was seen to persist even after six months of treatment.

*Meningeal Tuberculosis* : In meningeal tuberculosis the alterations noted were a moderate decrease in serum albumin and a slight increase in the gamma globulin content. The alpha<sub>a</sub> and beta globulins were increased in one case each. The changes in albumin and alpha<sub>2</sub> were gradual at the end of six months. The gamma globulins remained elevated. In the second case, which deteriorated clinically, the gamma globulin increased further and albumin levels lowered.

*Miliary Tuberculosis* : There was a marked fall in albumin, a marked increase in alpha<sub>2</sub> and a slight rise in beta fractions. All these returned to near normal limits after 6 months of treatment. However the markedly raised gamma globulin values did not regress.

*Lymphnode Tuberculosis* : The alterations observed initially in lymphnode tuberculosis were an increase in beta and gamma globulins. At the end of six months there was a gradual decrease in both the fractions. The albumin

TABLE 5  
Changes in serum protein fractions in patients—initial values

Type of disease	Total proteins Gm 5%	Percentage albumin	% alpha 1	% alpha 2	% Beta	% Gamma
Primary Tuberculosis	Range 5.6 to 6.8 Mean 6.3	50.17 to 60.24 58.43	2.98 to 4.24 3.76	7.34 to 11.33 7.73	6.85 to 11.67 8.91	19.02 to 20.64 20.43
Progressive primary Tuberculosis	Range 5.2 to 6.4 Mean 5.7	43.87 to 50.62 49.12	2.77 to 4.50 8.58	10.18 to 14.60 12.71	8.25 to 10.89 9.54	23.17 to 26.60 24.78
Chronic pulmonary Tuberculosis	Range 5.2 to 7.2 Mean 6.06	32.82 to 43.27 38.09	2.94 to 5.21 4.10	14.35 to 20.45 14.95	9.12 to 12.04 10.91	2 34.17 28.88
Abdominal Tuberculosis	Range 5.0 to 7.2 Mean 6.07	36.72 to 43.27 40.27	3.02 to 5.13 3.70	18.14 to 20.47 18.77	9.12 to 11.13 10.34	24.54 to 28.42 26.88
Protracted Hematogenous	Range 5.2 to 6.8 Mean 6.0	42.15 to 42.26 42.20	2.48 to 3.62 2.82	12.13 to 16.68 14.40	11.42 to 16.17 13.79	26.13 to 28.96 27.74
Miliary Tuberculosis Menigeal TB.	Range 5.4 to 5.9 Mean 5.63	38.78 to 43.72 41.05	3.07 to 4.65 3.80	16.75 to 21.79 19.35	9.31 to 13.85 12.11	20.53 to 28.19 23.67
Lymphnode Tuberculosis	Range 5.7 to 6.4 Mean 6.12	50.85 to 53.67 52.78	3.18 to 4.63 3.97	7.48 to 10.13 8.96	9.34 to 14.32 11.19	21.41 to 24.92 23.37

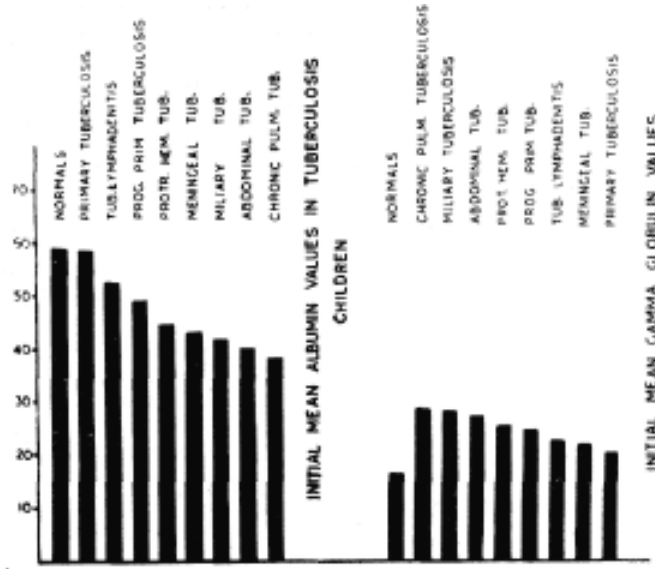


FIG. 1

Shows initial mean changes in Serum albumin and gamma globulin in patients

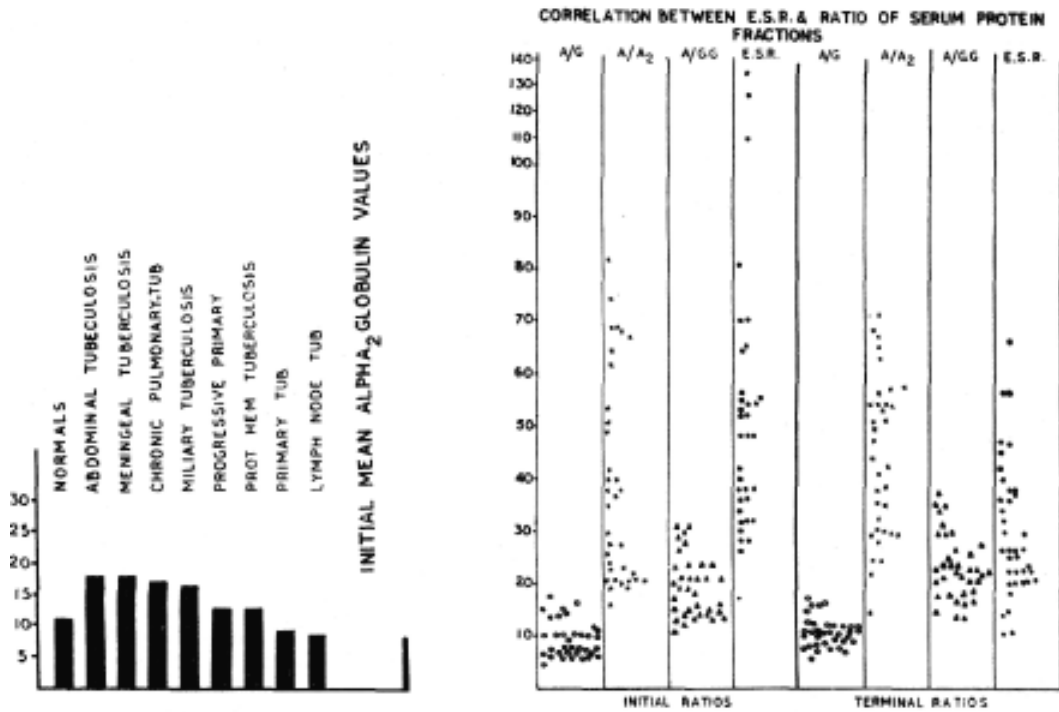


FIG- 2

Initial mean alpha, globulin Values in patient

FIG. 3  
Comparison Between E.S.R. and Various Serum protein ratios. Initially and at the end of six months

and alpha fractions were not affected in lymph-node tuberculosis.

*Serum Protein Fractions and ESR* • E S R was found to be more often affected. It was

observed that E.S.R. does not correspond with the albumin,  $\alpha_2$  or gamma globulin level either at the initial or final examinations. A considerable number of cases showed a disparity between E.S.R. and the ratios of protein fractions. On the whole, E.S.R. was abnormal in 34 cases whereas albumin/ $\alpha_2$  was low in 25 cases and the albumin/gamma globulin in 32 cases.

### Discussion

The present study was undertaken to determine the electrophoretic pattern of serum proteins in 35 cases of childhood tuberculosis. Our keenness to include cases meeting certain rigid criteria and only those on whom a six months' follow up was complete has necessarily restricted the number to 35 cases, but has the advantage of authenticity. In 24 cases the diagnosis was confirmed and in 11 cases it was tentative.

The normal values of serum proteins and their fractions obtained in our controls are compared with values obtained by other authors like Patel, Pohowala, and Gomez., who studied the electrophoretic patterns of serum proteins in normal children. None of the control cases showed abnormal values.

Total serum proteins did not show any significant alteration in tuberculous children. It is highly probable that the decrease in albumin content is compensated by an increase in the globulins thus leaving the total proteins virtually unaltered. Sirsi et al arrived at a similar conclusion on the basis of their experimental work.

Albumin fraction seems to be consistently lowered in cases of tuberculosis. Initially the reduction is more marked in patients with chronic extensive disease. The return of serum albumin to normal levels correlated well with clinical recovery, an observation in conformity with the work of Nemir et al. Lowered serum albumin levels in tuberculosis may be the result of associated under-nutrition and involvement of liver occasionally.

The  $\alpha_1$  fraction did not show any appreciable changes throughout the study. The  $\alpha_2$  globulins were found to be markedly increased in patients with meningeal, chronic pulmonary, abdominal and miliary tuberculosis. Nemir et al and Baldwin have recorded essentially identical observation.  $\alpha_2$  and beta globulins have been shown to possess anti-tuberculous properties and also growth inhibiting action on mycobacterium tuberculosis.

In the present study the beta globulins were found to be increased in only 5 out of 35 cases.

Nemir et al have also cited that beta globulin increase is not a constant feature, and a rise in beta globulin was associated with caseating forms of tuberculosis.

The gamma globulin fraction was found to be increased in all the 35 cases at initial examination and more or less related with the extent and activity of the disease with the exception of tuberculous meningitis. The rise of gamma globulin has been reported by many workers, and in general indicates good prognosis.

A good parallelism was detected between the extent of serum protein alterations and the extent of the clinical disease. Minimal changes were observed in minimal disease. In more advanced disease the albumin and  $\alpha_1$ , indicated moderate to marked changes except in case of meningitis. Similar findings have been expressed by Seibert, Baldwin, Volk and Gilliland et al. The changes observed in 24 cases, where tuberculosis was confirmed either by culture or by histopathological changes, were no way different from the 11 cases where tuberculosis was diagnosed on supportive evidence, even though the extent of changes differed. However, in these 11 cases also the values of serum albumin,  $\alpha_2$  and gamma globulin were slightly different from the values in controls.

Two other indices which were noted to be affected were the ratio of albumin/ $\alpha_2$  and albumin/gamma globulin. Their ratios were low initially in active disease, and attained near normalcy with clinical improvement.

No correlation whatsoever was noticed between the values of E.S.R., the levels of albumin,  $\alpha_2$  or gamma-globulin. Similarly, E.S.R. did not correlate with the albumin/globulin, albumin/ $\alpha_2$  or albumin/gamma globulin ratios. During clinical recovery E.S.R. decline and bio-chemical improvement did not go hand in hand. The lack of correlation is probably explainable on the basis that E.S.R. is dependent on many other factors like fibrinogen agglomerins and physical factors.

The above observations indicate that the serum protein alterations are not sufficiently constant as to be diagnostic of the various clinical types of tuberculosis though in general they correspond with the extent of the disease. On followup though improvement in the serum proteins fractions correlated with clinical improvement, they are still not as reliable or as sensitive as to replace periodic clinical assessment and radiological studies. Hence electrophoretic pattern of serum proteins cannot be recommended as a routine labo-

ratory procedure in childhood tuberculosis. More sensitive methods like study of immunoglobulins in each type of disease may help us to plug the lacunae in the knowledge of tuberculosis.

#### Summary and Conclusions

Thirty five children with various types of tuberculosis were studied clinically, radiologically, and serial determination of serum protein electrophoresis was done. The results were statistically analysed.

Changes were observed in serum albumin,  $\alpha_1$  and gamma globulin fraction in all types of childhood tuberculosis.  $\alpha_1$  and beta changes were not constant. The changes in serum protein fractions in the 24 confirmed cases and in the 11 cases with a tentative diagnosis of tuberculosis were essentially same, even though the degree of changes differed.

A good correlation was detected between the extent of the serum protein alteration on admission and the extent of the clinical disease. Albumin/ $\alpha_2$  ratio was more affected in advanced cases.

The return of albumin/ $\alpha_2$  ratio towards normal was found to be a good overall index of biochemical improvement.

There was complete lack of correlation between the alterations of E.S.R. and serum protein fractions or ratios.

The serum protein alterations observed in childhood tuberculosis are neither so constant nor so specific as to acquire any diagnostic significance.

More sensitive methods like study of immunoglobulins may be helpful.

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## HYPOGENESIS OF RIGHT LUNG ASSOCIATED WITH PULMONARY TUBERCULOSIS AND CORPULMONALE

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(From S.P. Medical College, Bikaner)

Hypoplasia or hypogenesis of lung is a congenital anomaly. Schinder (1912) classified the congenital anomalies of the lung into three types.

- (1) True aplasia or agenesis—Complete absence of the bronchi, lung tissue and vessels.
- (2) Aplsia—Bronchial bud is present but no lung tissue.
- (3) Hypoplasia or Hypogenesis—Bronchial bud is present but ends in poorly developed lung tissue.

The congenital anomaly of the lung is a very rare condition with reported incidence of 1 in 10,000 (Neterville, 1957), Ingram & co-workers (1950) reported two cases of Aplasia of one lung. Oymenda et al (1953) studied 73 cases of different varieties of mal-development of the lung; 48 were found during autopsy and 25 in the living condition. One case of hypogenesis of lung (diagnosed on autopsy) associated with pulmonary tuberculosis and corpulmonale is being reported.

### Case Report

H.R. 13 years male was admitted to the T.B. Hospital, Bikaner on 16.1.72 with complaints of cough and expectoration of 3 years duration. Intensity of the symptoms used to increase during the winter months. A dull fleating chest pain had been persisting for the last one year. Dyspnea gradually increased leading to the present state of orthopnea. Oedema first appeared at the ankle 2 months ago and became generalised later on with increasing anorexia and loss of weight.

### Physical Examination

The patient was poorly built, anaemic with cyanosis and clubbing. Neck veins were engorged and free fluid in abdomen was present. Pulse 108/minute regular and good in volume. Respiration rate was 26 minute, regular, abdominothoracic B.P. 96/80 mm of Hg.

### Examination of Chest

Right hemithorax was flattened and smaller.

On right side of the chest, intercostal spaces were narrowed with the crowding of the ribs, drooping of the right shoulder was observed along with the diminution of the movements. Trachea was shifted to the right. Apex beat was located an inch medial to the mid clavicular line in the left 4th intercostal space.

Percussion note was dull on the right side of the chest. Breath sounds were markedly diminished on the right but not so on the left. In the left interscapular region harsh vesicular breathing was audible. No adventitious sound were heard on the right side but a few crepitations on the left supra scapular region, were heard.

### Abdomen

It was distended with the engorged veins. A soft liver with rounded margin was felt 1/2" below the right inferior costal margin and was tenders. Evidence of ascitis was there.

### Investigations

Hematological routine investigations, urine and stool revealed no abnormality except haemoglobin concentration was 7 gm %.

### Skiagram Chest

Homogeneous opacity on right side with shift of trachea and mediastinum, to the same side (Photo. 1).

### Probable Diagnosis

A case of pulmonary tuberculosis with atelectasis of right lung or hypogenesis of right lung with corpulmonale. Special investigations like bronchography, bronchoscopy and angiography could not be carried out as the patient's general condition was very low.

### Treatment

Digitalis, diuretic, intermittent oxygen inhalation, crystalline penicilline and usual anti-tuberculosis drugs were administered. Inspire of all the efforts the general condition of the patient continued to deteriorate and he finally succumbed (after 72 hours of his admission). Postmortem examination was performed on the next day and a bronchogram

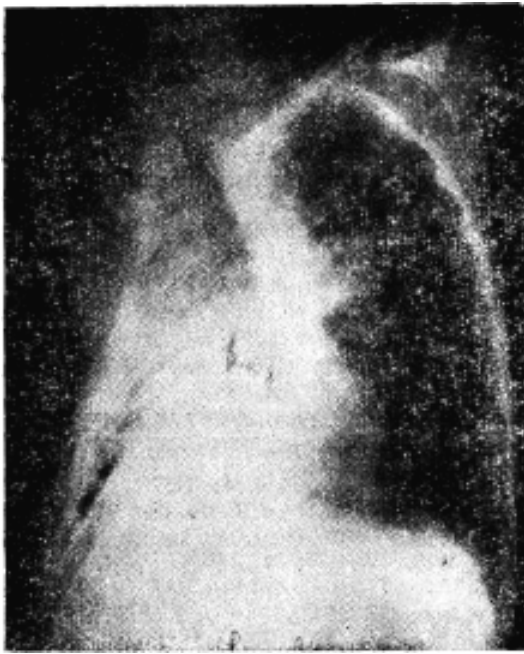


FIG. 1

was made from the autopsy specimen of the lung after injecting barium emulsion under pressure in the tracheo-bronchial tree (Photo. II). No abnormality was detected in any other organ.



FIG. 2

#### *Histopathological Report*

An under-development or hypo-development of the right lung tissue with diffuse scattered lesion in left lung histopathologically concomitant with pulmonary tuberculosis.

#### *Discussion*

This type of the congenital anomaly, because of its rarity provokes wide spread interest. Aetiological aspects of this developmental defect are still controversial. Different views are as follows.

I. These may be due to defects in the germ plasma (Steinberg et al, 1953, Nicks, 1957). Hence lung anomaly may be associated with other anomalies like patent ductus arteriosus, diaphragmatic hernia, tracheo-oesophageal fistula, oesophageal atresia, deformity of the ribs, vertebrae and agenesis of the abdominal organs. These associated findings, however, were absent in the case.

II. Genetic and foetal environment may have some role to play in the lung developmental defects (Wilson and Warkany, 1949). These authors produced congenital anomaly in the rat-off springs, by feeding mother-rats on vitamin A deficient diet.

III. Viral infection herpeszoster and chicken pox in pregnant mother have been reported (Field, 1946) to have led to this congenital anomaly in the off-springs.

These defects occur in the foetal life and lead to the cessation of development at its varying stages, resulting in multiform deformities from complete agenesis to partial defects in broncho-pulmonary system.

Mortality in these types of cases differs from person to person and depends upon the superadded infection (like in the present case) trauma stress and strain (Hochberg, Naclerio, 1955).

Life span has been found to vary from 15 to 72 years (Oymenda et al, 1953).

The symptomatology is extremely variable but acute symptoms of respiratory distress (as in the present case) are often absent.

Diagnosis depends upon the chest roentgenography tomography, contrast radiography (like bronchography and angiography) and bronchoscopy.

Differential diagnosis varies between (a) atelectasis, (b) fibrothorax and (c) diaphragmatic hernia.

Krishnamurthy et al, (1964) emphasised that the patient with maldevelopment or congenital

anomaly of the lung is very prone to develop chronic suppuration hence (like this case) leading to corpulmonale.

### Summary

The patient was admitted in corpulmonale and expired within three days. The clinical and radiological examination revealed a complete atelectasis of right lung with tubercular infiltration left upper zone leading to corpulmonale. There was suspicion of congenital anomaly like agenesis or hypoplasia of the right lung. The chest X-ray findings were corroborative but poor general condition of the patient prevented carrying out any further special investigations. The patient however could not survive and later on the postmortem examination revealed rudimentary (under developed) lung tissue attached to the right stem-bronchus.

Left lung showed small firm nodules scattered throughout the upper lobe. The bronchogram, made from the autopsy specimen revealed hypogenesis of the right lung which was confirmed

by histopathological examination of the specimen.

### ACKNOWLEDGEMENT

We are thankful to Dr. K.C. Gupta, Principal and Controller of S.P. Medical College and Associate Group of Hospitals, Bikaner, for his kind permission to publish this paper.

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## LOSS OF TUBERCULIN SENSITIVITY IN TUBERCULOUS PERITONITIS

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### Introduction

Negative tuberculin reaction has become a common finding in proved cases of tuberculosis and recently there have been a few reports of such finding in tuberculous peritonitis. The percentage of negativity has varied from 17-38 % in various reports. In the circumstances prevailing in India today, a negative reaction does have a vital bearing on the ultimate diagnosis of the disease. Many times such cases are likely to be misdiagnosed as cirrhosis with portal hypertension or nutritional hypoproteinemia when the exuded ascitic fluid has low total protein content; and associated negative tuberculin reaction would certainly favour a non tuberculous aetiology. Two case reports are presented here of tuberculous peritonitis, discussing the pattern of tuberculin sensitivity obtained.

### Case Reports

*Case No. 1:* A 30 year old female reported to hospital on 2nd May, 1971 with symptoms of pain in the lower abdomen with progressive distension of 3 months' duration. There was no history of fever at the onset of, symptoms or later. Disturbed bowel habits and loss of appetite were present. There was no cough.

On examination she was moderately nourished, there was distension of abdomen with circumferential measurement of 36" over the umbilicus. Minimal pedal oedema was present. The respiratory rate was 28/m. Examination of abdomen showed no venous collateral. There was no palpable abdominal viscera. Fluid thrill was present but shifting dullness not was easily demonstrable. There was vague diffuse tenderness on pressure on the abdomen.

Respiratory system examination revealed the presence of fluid on the left side. Examination of other systems was not further contributory.

Total WBC count was 9400/cm. with a differential count of P L E. ESR was 42m.m in 1 hour. Skiagram of chest showed pleural effusion with a level at the 5th rib. Ascitic fluid total protein was 2.97 Gm.% There was 3200 cells/cram with lymphocytic predomination. Peritoneal biopsy showed nonspecific chronic

inflammatory cellular infiltration. Pleural biopsy showed tuberculous granulation tissue.

Tuberculin test done on 8-5-71 showed 4x 2 mm induration to 1 TU, PPD. Intramuscular streptomycin 1 Gm. /daily and I.N.H. 300 mgm were given from 12-5-71 and on 16-6-71 when tuberculin test was repeated with the same P.P.D , it showed 10x14mms of induration.

### Case No. 2:

A 35 year old female was admitted on 31-1-71 with fourmonths' symptoms of swelling of the leg, distension of abdomen, and frequent watery stools. A month prior to admission she developed productive cough and dyspnea. There was no fever.

On examination she was very poorly nourished, gross anasarca was present, with moderate anaemia. Examination of abdomen showed minimal distension with fullness of the flanks, doughy feel, and freely moving fluid intra — abdominally. There was no palpable viscera or tenderness. Other systems did not reveal anything contributory, except for scattered rales at the base of right lung.

Investigations revealed total WBC count of 7200/cm.m with a differential count of P L E. HB was 45% and total RBC count was 2.8/m. cmm. ESR was 28 m.m/1 hour and blood urea was 20 mgm %. Serum total protein was 7Gm %. Ascitic fluid total protein was 1 Gm% : Cytology showed 2800 cells/cmm., which had predominantly lymphocytes. Skiagram of chest showed RT. basal bronchio-pneumonitis with enlarged right hilar glands. Sputum smear was negative for AFB. Peritoneal biopsy showed granulomatous lesion which was compatible with tuberculosis.

Tuberculin test was done, as in the first case, on 20-2-73. It was negative and there was no induration. She was given antituberculous therapy as in the first case along with prednisone 5 mgm bid; on repeating the tuberculin test on 11-3-73 it showed 2x4 mm and on 29-3-73 14x 16 mm of induration. The patient's general condition had improved at the time of the third tuberculin test in that

the anasarca disappeared giving her an emaciated look.

### Analysis

It is seen in these two cases that about 3 and 4 months after the onset of symptoms and signs of peritonitis the tuberculin sensitivity was negative. Treatment with antituberculous drugs produced "conversion" about a month after. The first patient was in moderately good health, when tuberculin test was carried out and found negative and there were none of the conditions as enumerated by Edward and Kent which could give rise to a false negative reaction. The second patient's general conditions did not improve significantly to account for the 'conversion' after treatment for a month.

### Discussion

Patterson had shown that when live and virulent mycobacteria were introduced into the pleural cavity of a guinea pig, which was not previously infected by these bacilli, it produced no effusion, but when same animals, sensitized to these bacilli by a previous vaccination were inoculated intrapleurally with attenuated or even killed mycobacteria, they developed effusion. Based on these observations he postulated that serous exudation in pleural tuberculosis was a manifestation of hypersensitivity to tuberculo-protein. This was widely accepted and it is conceivable that peritoneal membrane having embryological unity and histological identity to pleura should also have similar pathological response to this infection.

On this principle, it is presumed that the two cases reported above should have had a positive tuberculin reaction (a demonstration of hypersensitivity to tuberculo-protein) if tested at the onset of peritonitis, for serous exudation is only a consequence of the existing hypersensitivity. The negative reaction observed after 3&4 months of peritonitis and exudation, in the above two cases, are therefore examples of "reversion" of tuberculin reaction with the progress of disease. Though this reversion of tuberculin reaction is speculative in these instances, we have very convincing observation of this phenomenon in tuberculous pleural effusion.

It is believed that the spread of infection on the peritoneal surfaces, which involved a process of repeated inoculation as the spread occurred, was analogous to the frequent inoculations of avirulent bacilli done intradermally by Rich to demonstrate desensitization in a hypersensitive animal.

Recent studies have also shown that the reversion may be due to the loss of "thymus dependent" lymphocytes from the blood stream. It is common knowledge that the immune mechanism of an allergic individual responds with massive conglomeration of lymphocytes around a focus of tuberculous infection. With a multitude of spreading foci scattered over the peritoneal membranes, it is possible that there occurred an enormous loss of lymphocytes from the blood stream into the perifocal tissues. This temporary drop in the specific antibody carrying lymphocytes from the circulation may be the cause of reversion.

The two cases also showed conversion on treatment for about a month with antituberculous drugs—a paradox reported as far back as 1953 in tuberculous meningitis—irrespective of the general health of these patients, by Taylor et al. It is believed that the exhibition of antituberculous drugs prevented further spread of the infection, which permitted a reaccumulation of the antibodies in the system to give a rising titre and thence a positive reaction.

Recently, two cases of tuberculous peritonitis were followed up with serial WBC differential counts. It was noted that the lymphocyte counts in the peripheral blood, increased by 5 and 8 % respectively when conversion of the tuberculin reaction occurred, compared with the lymphocyte percentage obtained earlier, when there was negative reaction. This suggests that the conversion of tuberculin reaction on treatment, in exudative tuberculous infection of the peritoneum maybe due to the replenishment of the lymphocytes in the system after arrest of the disease.

### ACKNOWLEDGEMENT

I am thankful to the Superintendent, Government General Hospital, Madras, for according permission to publish this paper.

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## **LETTER TO THE EDITOR**

### **TUBERCULOSIS, OLD or PURIFIED**

Sir, Since childhood tuberculosis is widely prevalent in urban as well as rural Kerala we have got into the habit of carrying out the Mantoux test as a routine in our paediatric practice for early detection of primary tuberculosis.

Unfortunately, the National Tuberculosis Institute authorities do not think that the Mantoux test is a useful diagnostic aid under Indian conditions and we just cannot get the test done with Purified Tuberculin Rt 23 with Tween 80 (Danish) at the District T.B. Diagnostic Centres. We are using Old Tuberculin 1 in 1000 and in our experience O.T. made by the Human Institute for Sarobacteriological Production and Research, Budapest, Hungary gives satisfactory results. We now learn that there may not be fresh imports of this material for some time to come. To ascertain whether the other brands of Tuberculin available in the market are equally reliable we have lately been doing the Mantoux test in both the forearms of the child—the Hungarian O.T. 1 in 1000 on the left forearm and one of the other three listed below on the right forearm.

Group	Name	Manufactured by	Diluent
1.	Old Tuberculin, 1 in 1000	Serotherapi Inst. Vienna	N. Saline
2.	Old Tuberculin 1 in 1000	Bharat Labs, Bombay-7.	Sodium Chloride 0.05 gm Carbolic acid 10 drops Distilled water 100ml.
3.	Purified Tuberculin Rt 23 10 TU	Desai Labs, Athwa Lines, Surat-1.	Ready made solution.

So far we have carried out this comparative study in 60 children, 20 in each of the 3 groups shown above. It was most disturbing to find that while reactions exceeding 15 mm of induration were frequently noticed in the Lt. forearm (Hungarian O.T.), the corresponding induration in the Rt. forearm (Austrian, Bharat or Desai) was consistently minimal in the same children.

*Could it be that the low mantoux positivity found in Tuberculous children in India 2,3 is due partly to the variable quality and potency of Tuberculins available in our Country?*

We wonder how Desai's Purified Tuberculin RT 23 will compare with the Danish purified Tuberculin RT 23 distributed by WHO over most parts of the world but not made available to paediatricians in private practice.

*We Paediatricians working in the community badly need advice regarding the availability of reliable brands of Tuberculin (old or purified) in this country. Would not our eminent medical scientists take up the challenge and come to our aid through the columns of esteemed journal?*

Yours etc,  
**K.P.B. Nair**  
Naikenal, Trichur  
Kerala

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## CASE REPORTS

### TUBERCULAR OSTEOMYELITIS OF SKULL BONES

D. RAJA REDDY, SUGUNA RAMMOHAN, A. KRISHNA CHARI AND K. SATYANARAYANA  
(From Gandhi Medical College, Hyderabad, A.P.)

Tubercular osteomyelitis of skull bones is very rare. The incidence of calvarial involvement by tuberculosis varies and is estimated to be about 0.2 % of all forms of bony disease. Even though tuberculosis is rampant in our country including its affection of the nervous system, there is paucity of proven cases of skull bone involvement and to the best of our knowledge only four cases are on record.

#### Case Report

I.E., a 55 year old married housewife, was admitted to the Neuro-surgery unit on 23-2-1973 with a discharging sinus over the left forehead. About three months prior to admission patient sustained a minor injury over the left frontal region which resulted in a tender swelling. A few days later she developed low grade fever and the swelling increased in size. A few weeks later, a discharging sinus ensued from the centre of the swelling. Patient later admitted to having a wound over the left supraclavicular region a few months before the present illness which, however, healed without any treatment.

Physical examination revealed a healthy middle aged lady who had a painless firm swelling 3" X 2" arising from the left frontal bone. There was a sinus in centre of the swelling with undermined unhealthy edges which oozed thin serous fluid. No lymph nodal enlargement could be detected. General systemic and neurological examination did not reveal any abnormality.

Urine analysis, complete blood picture, fasting blood sugar, serum tests for syphilis and chest skiagram were reported as normal. Erythrocyte sedimentation rate was 55 mm for the first hour by Westergren's method. Radiographs of skull revealed circumscribed osteolytic lesion in the left frontal region with typical 'bone sand' appearance suggestive of osteomyelitis with sequestration. There was sclerosis of the adjoining bone (Figs. 1 and 2).

Patient was operated upon on 28-2-1973. A left frontal scalp flap was turned centering over the swelling. The mass was yellowish grey in colour. Both tables of skull were eroded with extensive spread of the infection into the epidural space. The duramater appeared



FIG. 1

Antero-posterior view of skull skiagram showing typical 'bone sand' appearance within the osteolytic lesion

thickened. Most of the swelling including epidural component was removed. Postoperatively patient was given antitubercular drugs. She remains asymptomatic when last seen four months after the operation.

*Pathology report:* The biopsy material consisted mostly of irregular bits of soft tissue. Histopathological examination revealed minute focal areas of caseation surrounded by epithelioid and giant cells with granulation tissue, infiltrated with lymphocytes, plasma cells and mononuclear cells (Fig.3). In some areas bony spicules were seen. Diagnosis was tubercular osteomyelitis.

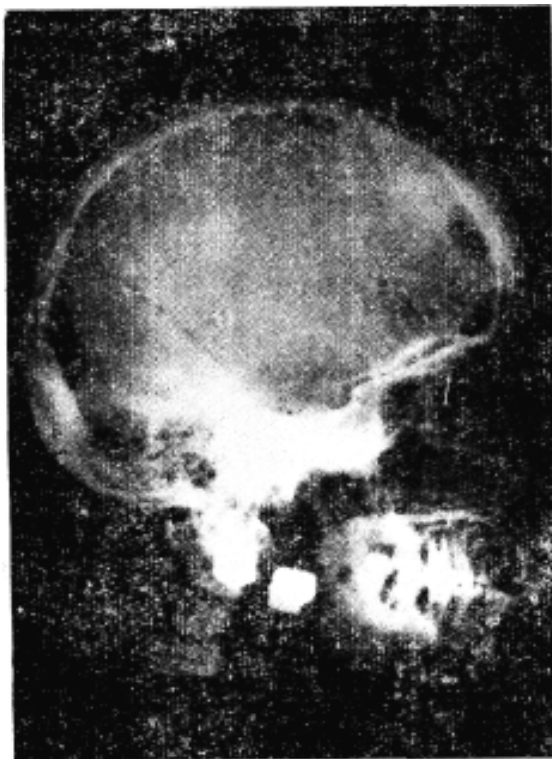


FIG. 2

Lateral view of skull skiagram showing osteolytic lesion with sclerosis of the adjoining frontal bone

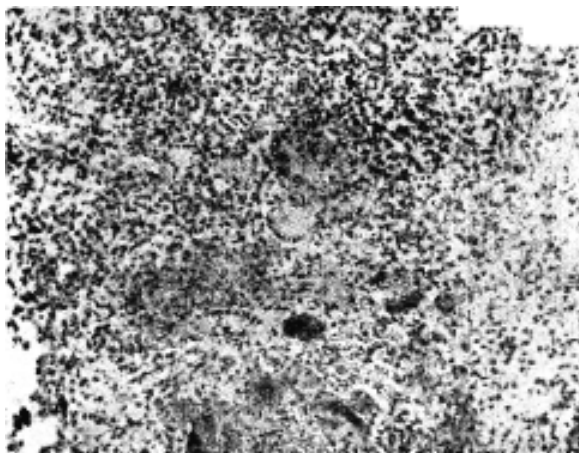


FIG. 3

Photomicrograph of the biopsy showing features of tuberculosis. H.E. X 200

### Discussion

Review of literature reveals that 75-80% of

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all cases of tub; "culosis of skull occurred before the age of 20 years and were more common in males. Trauma has been suggested as playing a role in the genesis of the disease and it is interesting to note that in the case reported here trauma which preceded the illness seemed to have localized the disease to the left frontal region. Majority of the cases of calvarial osteomyelitis had primary focus of tuberculosis elsewhere in the body but in a number of cases with solitary skull lesion no other lesion was found. The latter cases in the absence of primary focus were considered to have primary skull tuberculosis. In this case there was a healed lymph node lesion in the left side of neck which could have been tubercular in nature.

The haematogenous focus of infection in the skull bones starts in the diploe. Frontal and parietal bones are commonly affected. Infection may spread through the outer table and may present as a subgaleal mass. The firm bony mass in the early stages can be mistaken for a bony tumour either primary or secondary. Later it breaks through the skin and forms 2. sinus. Infection may erode the inner table and may spread extensively in the epidural space. Dura usually forms a good protective barrier to the spread of infection to the meninges and brain. Depending upon the individual resistance two types of lesions are described, circumscribed or punched out type and a diffuse infiltrating type.

Radiological findings are not characteristic of tubercular osteomyelitis. In early stages an area of decalcification will be seen which will progress as disease spreads. If a circumscribed lesion develops, clearly demarcated osteolytic lesion may be surrounded by a thin area of increased density. The differential diagnosis consists of intradiploic epidermoid, haemangioma, histiocytosis X, osteoid osteoma etc. If there is a progression of the lesion, irregular bony defect with either 'bone sand' or actual sequestrae may be demonstrated. If there is superadded secondary infection, new bone formation may be seen.

In early stages of this disease, antitubercular treatment may alone suffice but in later stages with large swelling and sequestration of bone, surgical removal of all disease area will be necessary.

### Summary

A case of osteomyelitis of skull bones due to tuberculosis in a middle aged lady is reported. Literature is briefly reviewed.

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## NEWS AND NOTES

### 25TH TB SEAL SALE CAMPAIGN

The 25th TB Seal Sale Campaign was inaugurated in India on 2nd October, 1974. President Shri Fakhruddin Ali Ahmed in a special message appealed to the people to participate in the campaign to strengthen the hands of the Tuberculosis Associations. Shri S. Ranganathan, M.P., President of the Association in a message hoped that the 25th Campaign will be a resounding success both in strengthening the national front against tuberculosis and in collecting funds for promoting voluntary work. The campaign will terminate on 26th January, 1974, the Republic Day. The new Seal design carries the motif 'A Dove in Flight'.

### EASTERN REGION CONFERENCE

The ninth TB Conference of the Eastern Region of the International Union Against TB and the 22th National Conference on TB & Chest Diseases will be jointly held in New Delhi from the 4th to 8th November. The Conference will be inaugurated by Sri Fakhruddin Ali Ahmed, President of India.

### REFRESHER COURSE IN TUBERCULOSIS

The New Delhi TB Centre organised a short-term Refresher Course in Tuberculosis for the benefit of medical practitioners outside Delhi. The Course was held from 28th to 30th October, 1974. Ten medical practitioners participated.

### SEMINAR AND SHIBIRS

The Karnataka State TB Association organised TB Shibirs at Mysore, Bangalore,

Gouribidanur and Chintamani Taluks (Kolar District) from 11th to 31st August, 1974. The Shibirs evoked good response and BCG Vaccination and case detections were successfully implemented.

The TB Association of Kerala organised a Seminar for High School teachers in Trivandrum district on the occasion of the Silver Jubilee celebrations of the Seal Sale Campaign. The Association in collaboration with the Health Services Education Bureau also organised a 3-day Exhibition for educating the public on Tuberculosis and allied matters.

Under the joint auspices of the TB Association of Andhra Pradesh and Rotary Club of Sirpur-Kagaznagar a Tuberculosis Check-up Camp was held in the premises of Govt. E.S.I. Hospital, Sirpur-Kagaznagar on 8th October 1974. Shri Kodati Rajamallu, Minister for Medical & Health, Andhra Pradesh, inaugurated the Camp.

### HAND BOOK ON TUBERCULOSIS

The Hand Book on Tuberculosis by Dr. S.P. Pamra (Director, New Delhi TB Centre) is now available in Hindi. The Hindi edition has been published by the U.P. Tuberculosis Association. The book provides in simple language information that para-medical students should know about tuberculosis such as the clinical features of the disease, the present philosophy of Tuberculosis control and treating patients as quickly as possible in their own homes. Copies can be had from the U.P. Tuberculosis Association, 1 A.P. Sen Road, Lucknow, on payment of Rs. 2.50 plus postage.

# The Indian Journal of Tuberculosis

## ABSTRACTS

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### **Controlled clinical trial of four short-course (6 months) regimens of chemotherapy for treatment of pulmonary tuberculosis**

*Second Report; East African British Medical Research Council. The Lancet: 1973, 1, 1331.*

A total of 1137 patients were included in the trial from 27 centres in 3 East and Central African countries between April, 1970 and September, 1971. The patients were divided into 5 groups. One group was put on streptomycin, INH and Rifampicin daily; another on streptomycin, INH and pyrazinamide; third on streptomycin, INH and thiacetazone; fourth was given only streptomycin and INH and the fifth group was given INH and thiacetazone daily with streptomycin for the first 8 weeks. The duration of treatment was 6 months only in the first four groups and 18 months in the fifth group (standard regimen). The results were analysed at 6 and 18 months after the start of treatment. At 6 months only 4 patients (one on streptomycin plus INH and three on standard regimen) were failures of chemotherapy. There was very little drug toxicity. The bacteriological relapse rate between 6 and 18 months were 2% in the Rifampicin regimen, 10% in the pyrazinamide regimen; 22% in the thiacetazone regimen, 27% in the streptomycin, INH regimen and 3% in the standard regimen. Most of the relapse occurred by 12 months and nearly every patient who relapsed did so with drug sensitive bacilli. It is concluded that the Rifampicin regimen is highly effective even when given for 2 months despite the severity of disease and that the pyrazinamide regimen approached it in efficacy. Both these regimens compare very favourably with standard 18 month regimen.

S.P.P.

### **Evaluation of closely supervised chemoprophylaxis**

*F. Van Der Kuyp, P.B. Barlow & J.B. Stockkn. Amer. Rev. Resp. Dis.; 1973, 107, 1087.*

The study is based on INH prophylaxis of 3,423 persons treated from a clinic with monthly

supervision. 60.6% completed one year's treatment; 70.5% completed 6 months or more. Premature stopping of treatment was due to lack of co-operation in 21.3%, side effects in 10.3% and the remaining for a variety of other reasons. The most frequent side effects were pruritic rash (2.2%) gastro-intestinal symptoms (2.8%) and dizziness (1.6%). SGOT was higher than 199 units in 0.8%. There were 5 cases of overt hepatitis with one death. In others, the symptoms and abnormal laboratory findings subsided after the drug was discontinued.

S.P.P.

### **Characteristics of 105 tuberculosis patients who reactivated in the city of St. Louis**

*W.C. Banton et al. Amer. Rev. Resp. Dis.; 1973, 107, 1119.*

From January 1968 to December 1971, 2,375 arrested tuberculous patients were followed up and the characteristics of 105 amongst them who had a relapse during this period were critically evaluated. Of the 105, 16 relapses were identified post-mortem. Of the remaining 89, 70 or 78.7% were 40 years of age or older, 63 or 70.8% were men and 50 or 56.2% were whites. Only 15% of the cases were minimal. The time of sputum conversion appeared to have no effect on probability of relapse. Thirty seven or 41.6% of relapses were discovered on routine check up at the clinic and the remaining attended the clinic because of chest symptoms. The presence of associated diseases appear to have little effect. Twenty three or 25.8% only had associated problem like alcoholism, mental disease, drug addiction or chronic chest diseases. 67.4% of the patients had been treated at the health centres, 15.7% private practitioners and 10.1% in hospitals. 12 or 13.5% of the relapsed cases had a complete uninterrupted course of treatment. The treatment of 7 out of these 12 was completed 5 years earlier and was with INH and PAS only. Of those patients with incomplete treatment, 15 or 16.9% had been regular, 11 or 12.3% irregular and 2 or 2.2% had stopped the treatment against medical advice. The characteristics of

the 16 cases of relapse detected after death did not differ significantly from the other 89 except that the time of sputum conversion was 13 months or longer for 9 cases or 56% and none of them had minimal disease. The authors conclude that majority of the persons who take 24 months of effective chemotherapy regularly do not reactivate. Men 40 years or more in age with moderately or far advanced disease constitute the high risk group.

S.P.P.

#### **Tuberculosis in 84 BCG vaccinated young adults**

*B. Groth Petersen. Amer. Rev. Resp. Dis. 1973, 107, 1119.*

84 cases of pulmonary tuberculosis amongst previously BCG vaccinated young adults showed the disease to be similar to the pattern found in un-vaccinated persons in respect of clinical features, bacillarity, extent and localization of lesions and presence of cavities. These 84 cases were found in the period 1964 to 1969 amongst 140,000 persons vaccinated in Copenhagen. They were 15 to 34 years of age; 37 were men and 47 women. 70% were sputum positive, the bacilli being of human type. None of the cases had characteristic "primary manifestations." None had miliary of meningial tuberculosis. However, 3 had serous pleurisy. 31 % had cavities. 40 of the 84 patients gave a history of close contact with an infectious tuberculous patient. In 20 the contact was within the last one year.

S.P.P.

#### **BCG Vaccination and Leukemia Mortality**

*S.R. Rosenthal et al, J.A.M.A.; 1972, 222, 1543.*

55,414 Negro infants in Chicago were vaccinated at birth with BCG. There were 172,986 unvaccinated infants in the same population. The Board of Health Statistics were reviewed for death due to leukemia upto the age of 6 years in these children. One leukemia death was reported in the vaccinated group, a rate of 0.31 per 100,000 per year. Twenty one deaths occurred among unvaccinated infants, a rate of 2.02 per 100,003 psyear. The difference between these rates is significant ( $P=0.04$ ).

S.P.P.

#### **Effect of BCG on carcinogen-induced tumor development in mice**

*T. Kataoka et al. Japan, J. Med. Sci. Biol.: 1972, 25, 377.*

Immunoprophylactic effect of BCG against tumor development in mice has been studied,

BCG vaccination was carried out before and after msthylocholanthrene (MCA) and N-nitrosodiethylamine (DEN). The data indicate that the BCG has some inhibitory effect on the development of tumor induced by the two carcinogens, MCA and DEN, although the cumulative tumor incidence eventually reaches the level in control animals. This inhibitory effect has been attributed to a non-specific stimulation of the immune response by BCG leading to a more efficient suppression of antigenic tumor cells. BCG also causes a change in the rate of macrophage activation e.g. increased lysosomal contents and bactericidal activity of macrophages.

S.P.P.,

#### **Characteristics of African Mycobacterium Tuberculosis**

*M.P. Zykov, H. Roulet and N. Gava. Amer. Rev. Resp. Dis.; 1973, 108, 93.*

When mycobacterium tuberculosis isolated from patients was passed through guinea pigs, drug resistance occurred among originally susceptible cultures and restoration of susceptibility occurred in originally resistant strains as well. These changes were not identical for all drugs. Reversion was most frequent and spontaneous resistance most common with thiacetazone. Neither process depended on catalase activity. An essential difference was found in the frequency of reversion to susceptibility of cultures resistant to PAS and among catalase positive and catalase negative strains. The preponderance of reversion over spontaneous resistance to streptomycin was found regardless of catalase activity. Changes in the susceptibility to TNH as the result of a single passage in guinea pigs occurred most rarely and were not connected with changes in catalase activity. After guinea pigs passage of catalase negative cultures, restoration of catalase activity occurred in 36.2% of cultures. Restoration of catalase activity was not associated with loss of resistance to INH, which occurred in only 3.4% of cultures. In the catalase negative cultures, an initial high degree of INH resistance was retained in 93.2% after passage through guinea pigs, whereas the percentage among catalase positive strains was only 41.3%.

S.P.P.

#### **Virulence attenuation and other biological modifications in multiple drug resistant tubercle mycobacteria**

*G. Daddi et al. Estratto dal—Boll Ist Sieroter, Milanese; 4, 50.*

Strains of tubercle bacillus after becoming

resistant to 3 or more drugs showed modification of biological characters also. Their virulence becomes considerably reduced especially for the guinea-pigs. This phenomenon no longer seems related only to INH resistance, as was the case in the past. It becomes evident in the case of resistance to other drugs also, more particularly Rifampicin.

S.P.P.

#### **Effect of radiation on microbiological characteristics of M. Tuberculosis**

*M.P. Zack, K. Stottmeier, G. Berg and H. Kazemi. Amer. Rev. Resp. Dis.; 1973, 107, 1088.*

Pulmonary tuberculosis with primary drug resistant organisms developed in 2 patients after radio therapy. The role of radiation on mutation expressing itself as drug resistance and/or changed viability was, therefore, studied *in vitro*. It was found that the viability began to decrease at radiation levels of 1000 rads and decreased linearly with higher levels of radiation. Three of the 42 radiated culture developed drug resistant organisms (one each to INA, PAS and streptomycin). Occurrence of this drug resistance was clinically significant.

S.P.P.

#### **The incidence of positive urine culture for M. Tuberculosis in a general tuberculosis patient population**

*R.R. Bentz et al. Amer. Rev. Resp. Dis.; 1972, 107, 1086.*

Urine of 238 patients of active tuberculosis was cultured for AFB as a routine measure from 1966 to 1970. The culture was found positive in 33 patients. Of these 33 patients, 10 had clinical evidence suggestive of renal tuberculosis and one was found to have prostatic lesions at prostatectomy. In the remaining 22, genitourinary tuberculosis was not suspected till the positive culture was found. Of these 22 patients, 13 were cases of pulmonary tuberculosis and 9 had extra-pulmonary disease other than genitourinary. Many patients with positive urine cultures had no abnormality on examination of urine or I.V.P.

S.P.P.

#### **A profile of the new active tuberculosis patient in Harlem**

*J.G. Collins. Amer. Rev. Resp. Dis.; 1973, 107, 1121.*

Characteristics of all new patients reporting

during a 6 month period in 1970 have been analysed in view of the high new case rate in this area (135 per 100,000). Only 12.5% of the new cases were married, 80 % were single. 50% did not have any household contact. The highest incidence was amongst patients located in the centre of the district with the rate for areas on the outer perimeter of the district being significantly lower. 75% of the patients had lived in the district for 10 or more years; however they had no permanent home and had moved their residence several times during the previous 10 year period. More than 80% of the patients had one or more significant problems other than tuberculosis which required medical care during the 5 years period before diagnosis of tuberculosis.

S.P.P.

#### **Correlation of lymphocyte transformation with tuberculin skin test sensitivity**

*S.D. Miller and H.E. Jones. Amer. Rev. Resp. Dis.; 1973, 107, 530.*

The degree of delayed hypersensitivity to tuberculin purified protein derivative (PPD) was determined in 34 subjects by sequential skin testing with first, intermediate and second strength PPD, and simultaneously the subjects' lymphocytes were maximally challenged *in vitro* with PPD.

A linear relationship existed between the minimal strength of tuberculin required to detect skin test sensitivity and the logarithm of the lymphocyte transformation index. In 25 of the 34 subjects, the size of the skin test response to any strength of tuberculin could be predicted within one standard deviation from the degree of lymphocyte transformation. The occurrence of some degree of lymphocyte transformation in all subjects who manifested skin test responses of 1 to 4 mm and 5 to 9 mm to some strength PPD compared to no lymphocyte transformation in subjects completely non reactive to the 3 strengths of tuberculin suggested that most skin test reactions, regardless of size, have an *in vitro*, immunologic correlate.

These data suggest that small skin test reactions i.e. 1 to 4 mm, although not reactive by the established epidemiologic criteria are significant immunologic events. This concept might help explain the booster effect that occurs occasionally with tuberculin test and was observed in 2 of the 34 subjects in this study.

Lymphocytes transformation might offer an additional method of assessing patients for

tuberculin hypersensitivity that avoids the potential booster effect seen with skin testing

S.P.P.

### **Pulmonary Manifestations in Leukemia**

*Tadafumi Hagihara et al. Materia Medico Polona; 1972, 4, 176.*

A comparative study of the pulmonary manifestations associated with leukemia in 132 clinical cases and 84 autopsy cases in the department of Internal Medicine, Ninon University, Tokyo has been reported. It was found that such manifestations are apt to develop more in the terminal stages of the disease and, therefore, are demonstratable more frequently in the autopsied lungs than in the skiagrams of the chest (80% in the former against 25% in the latter). The manifestations most often found were leukemic infiltrations, hemorrhage, infection (bacterial, fungal, tuberculosis etc.) infarction, hilar mediastinal lymphadenopathy and pleural effusion. Differential diagnosis is often difficult since the abnormal shadows observed in these cases were different from what they usually look like. This modification in appearance is due to toxic factors of leukemic cells, the effects of anti-leukemic agents and immunosuppression. Leukemic infiltration and hemorrhage were present in about half the cases and the other manifestations were seen much less often. Bacterial pneumonitis developed either hematogenously or bronchogenically. In the former the lesions tended to be bilateral and diffuse whereas in the latter, the lesion was in many cases unilateral and localized. Fungi most often encountered were *Candida* and *Cryptococcus*. In one case aspergilloma was present-

S.P.P.

### **Aspiration biopsy in diagnosis of pulmonary nodule**

*James V. Zelch, Anthony F. Lalli, Lawrence J. McCormack & Doris M. Belovich. Chest; 1973, 63, 149.*

Percutaneous pulmonary biopsy is a direct approach to diagnosis of a pulmonary nodule. The results of 208 consecutive pulmonary aspirations have been reported. The accuracy of diagnosis obtained was 93%. In 105 out of 124 cases of carcinoma, the specific cell type could also be established.

The procedure is quick and easy, can be utilized on an out-patient basis and can save

valuable time. Overall complication rate was 18% with no serious morbidity or mortality.

S.P.P.

### **Biopsy of non-palpable scalene lymph nodes in carcinoma of the lung**

*John W. Brantigan, Charles O. Brantigan and Otta C. Brantigan. Amer Rev. Resp. Dis.; 1973, 107 962.*

Bilateral scalene node biopsies were done in 341 consecutive patients with histologically proved carcinoma of the lung in Baltimore. Biopsies were positive in 51 out of 55 patients with palpable nodes and in 68 out of 286 (23.8%) with non-palpable nodes. Two patients had minor complications after biopsy (Pneumothorax and wound infection in one each). In another patient who was inadvertently given anticoagulant therapy, ecchymosis developed at the biopsy site but cleared very quickly. There were no major complications. Although biopsy of palpable node is a standard procedure, routine biopsy even if the nodes are non-palpable is a valuable procedure since involvement of the scalene node indicates inoperability of the new growth. Biopsy of the node on ipsilateral side alone is not enough. Biopsy should be bilateral in all patients in whom surgery is being considered.

S.P.P.

### **Cytology in diagnosis of cancer affecting the lung**

*Ute Wagner Rosa, Joao Curios Prolla & Eleoner da Silva Gastal. Chest; 1973, 63, 203.*

One thousand consecutive patients were subjected to sputum cytology in a chest hospital in Brazil from 1965 to 1971. There were 381 patients of carcinoma and a positive cytologic diagnosis was achieved in 71%. Specificity was 99%- Results were better in central tumors (82.5%) than in peripheral tumors (48%) or metastases (62%). The results were better for sputum cytology than for bronchial lavage aspirates (42.5%) and were best for epidermoid carcinoma (84.5%).

Best results in central tumors were achieved by bronchial biopsy and best results in peripheral tumors were obtained by transcutaneous needle biopsy. In metastases, cytology and needle biopsy gave similar results. Bronchial biopsy was useless in peripheral tumors and in metastases that were not endo-bronchial and centrally situated.

S.P.P.

### **A controlled trial of prednisone treatment of sarcoidosis**

*Harold L. Israel, D.W. Fontz and Robert A. Beggs. Atner. Rev. Resp. Dis.; 1973, 107, 609.*

Ninety patients with sarcoidosis were enrolled in a randomized trial of the effect of three months of prednisone therapy. Eighty three patients completed treatment and were followed for at least a year. In thirty seven patients with hilar lymphadenopathy alone on admission to the study, no significant difference between treated and control groups was noted either at the end of the treatment period or after a mean interval of 5.2 years. In forty six patients with pulmonary infiltration, significant improvement was evident at the end of the treatment period but no differences between treated patients and controls were demonstrable after a mean interval of 5.4 years.

Subsequent relapse or progression necessitated prednisone therapy in 38 per cent of patients receiving placebo and 24 per cent of treated patients. The prognosis was significantly better in patients entering the study with hilar adenopathy alone. These observations support the views widely but not universally held, that prednisone exerts little influence on the eventual outcome of sarcoidosis but is ameliorative in patients with pulmonary involvement.

S.P.P.

### **Connective tissue damage in emphysema**

*W.G. Johanson, Jr., R.C. Reynolds, T.C. Scott and A.K. Pierce. Amer. Rev. Resp. Dis.; 1973, 107, 589.*

A study was planned to clarify the effect of papain on rat lung connective tissue by electron microscopy. Rats were exposed to an aerosol of 10 per cent papain for 4 hours, and their lungs were examined at intervals from immediately to 6 months after exposure. Papain selectively attacked the amorphous component of elastic fibres, leaving the microfibrils intact. Collagen was not altered histologically. Connective tissue appeared normal in animals examined later than 4 weeks after exposure.

A pathogenic mechanism for the development of emphysema is proposed that involves the acute dissolution of elastin by proteolytic enzymes, allowing structural remodeling of the lung, with subsequent regeneration of the persisting microfibrillar skeleton. This concept would unify morphologic observations with the

biochemical observations that connective tissues are not altered in emphysema.

S.P.P.

### **The variable effect of smoking on pulmonary function**

*Abraham S. Kuperman and Jeffrey B. Riker. Chest; 1973, 63, 655.*

The variable effect of cigarette smoking in airway obstruction was evaluated in 925 subjects who were tested in a respiratory disease screening programme in Albert Einstein Medical College, New York city. Five hundred and eighty nine amongst them were smokers who were divided into four groups in respect of their smoking habits. The flow rates in the group with very little smoking were indistinguishable from those in non-smokers. With increasing cigarette smoking there was a progressive reduction in mean flow rates and an increase in the incidence of severe obstruction. Nevertheless, a significant number of heavy smokers remained within normal limits. In the group with maximum cigarette smoking FEV<sub>1</sub>/VC was within normal limits in 29% and MMF was within normal limits in 21%. The highly variable individual susceptibility to the effects of cigarette smoking would seem to imply that other genetic and environmental factors are also operative in the production of airway obstruction. Identification of these additional factors would permit recognition of a high risk population.

S.P.P.

### **The current status of Serologic immunologic and skin tests in the diagnosis of pulmonary mycoses**

*Report of the Committee on Fungus Diseases and Clinical Diagnosis of the American College of Chest Physicians. Chest; 1973, 63, 259.*

In Actinomycosis, skin tests are of no value. Agar gel double diffusion test is positive in high percentage of patients with disseminated disease but is negative in localized forms. It has cross reaction with the tuberculosis. Fluorescent antibody technique is helpful in detection and identification of organisms in cultures and smears of tissue and exudates.

In Aspergillosis, skin tests are helpful in allergic aspergillosis but are of no value in aspergilloma and invasive type of disease, Agar gel and complement fixation tests are virtually diagnostic, when positive in aspergilloma and allergic aspergillosis but are of no value in

invasive type. Indirect fluorescent antibody test is of no apparent value.

In Candidiasis, skin tests are of no value. Precipitating and agglutinating antibodies are strongly suggestive of systemic candidiasis. Precipitating antibodies are not found in healthy persons but agglutinating antibodies are present in low titres in normal persons and patients with superficial infections.

In Coccidioidomycosis skin tests are of limited value. Agar gel and complement fixation tests are diagnostic if positive though negative reactions do not exclude it. Cross reaction occurs with histoplasmosis. Rising or falling titres are of prognostic value. Complement fixation test is useful in the diagnosis of meningeal disease. Fluorescent antibody test is useful in detection and identification of organisms in cultures, smears and formalin fixed tissues.

In Cryptococcosis, value of skin test is still under investigation. Indirect fluorescent antibody and tube agglutination tests are diagnostic if positive but of no value if negative. False positive reactions may also occur.

In Histoplasmosis, diagnostic value of skin test is extremely limited in adults except in certain selected situations. Complement fixation test is diagnostic if positive in high or rising titres but does not exclude Histoplasmosis if negative. Agar gel test is also diagnostic if positive but has cross reaction with Coccidiomycosis.

In Nocardiosis, the value of skin test is underdetermined and serologic tests are of no value.

S.P.P.

#### **Comparison of two regimens of simplified two stage chemotherapy in previously untreated pulmonary Tuberculosis**

*Newton Bethlein, Germano Gerhardt Fo, and Sergio Magaro. Tub. (1973), 54, 180.*

59 patients with untreated pulmonary tuberculosis and sputum positive on direct microscopy were allocated at random to 2 treatment regimens.

1. *SPH/SHOW*:—streptomycin 1 gm, isoniazid 300 mgm, PAS 15 gm in single dose daily in hospital for 12 weeks followed by streptomycin 1 gm and isoniazid 500 mgm once weekly under supervision for 40 weeks.

2. *SPH/H*: as in other regimens for the first 12 weeks, followed by self administration of 600 mgm isoniazid in a single dose daily.

Of the total 59 patients admitted to the trial, 17 had to be excluded (6 had isoniazid resistant cultures before treatment, 10 were lost to observation and one died during the first 12 weeks). At the end of 52 weeks, 14 (17 per cent) of the 19 patients in the *SPH/SHOW* group had bacteriological conversion compared with 21 (91 per cent) of the 23 in *SPH/H* groups. 5 (26 per cent) in the *SPH/SHOW* group and 2 (9 per cent) in the *SPH/H* group had unfavourable results. The results were not significant.

(H.B.D.)

#### **Co-operative controlled trial of a standard regimen of streptomycin, PAS and Isoniazid and three alternative regimens of chemotherapy in Britain**

*A report from the British Medical Research Council. Tub (1973) 5, 4, 99.*

The results at one year of a controlled clinical trial of three alternative regimens to the standard regimen of Isoniazid plus PAS with an initial streptomycin supplement are presented in 481 newly diagnosed sputum positive tuberclosis who were treated for three months in hospital followed by continuous chemotherapy for nine months as out patients. The cases were allocated at random to a) streptomycin plus isoniazid plus PAS followed by daily self administered isoniazid plus PAS (P. series), b) streptomycin plus isoniazid plus ethambutol daily followed by daily self administered isoniazid plus ethambutol (E. Series), c) streptomycin plus isoniazid plus rifampicin daily followed by daily self administered rifampicin, (R. Series), d) streptomycin plus isoniazid plus PAS daily, followed by twice weekly streptomycin plus high dosage isoniazid (plus pyridoxine) under supervision (S<sub>2</sub>H<sub>2</sub> series). The triple combination was given for 3 months in all 4 regimens. Of the total of 481 patients, 412 (109 P, 97 E, 103 R and 103 S<sub>3</sub>H<sub>2</sub>) were included for comparison.

At 12 months, only 2 of 101 P, 1 of 91 E, 0 of 85 R and 1 of 95 S<sub>2</sub>H<sub>2</sub> had an unfavourable bacteriological response. In subsidiary comparison of patients who either continued on their allocated regimens throughout or had chemotherapy changed for bacteriological relapse, 3 of the 82 P, 9 of 73 E, 0 of 85 R and 1 of 57 S<sub>3</sub>H<sub>2</sub> patients had an unfavourable bacteriological outcome at 12 months.

Bacteriological conversion on sputum smear

examination was similar in the four series but in the cultures was significantly more rapid in the R patients than in the other three series. Of the 466 (122 P, 116 E, 112 R, 116 S<sub>2</sub>H<sub>2</sub>) patients eligible for comparison of the side effects, 30 per cent of the P, 21 per cent of the E, 21 per cent of the R and 32 per cent of the S<sub>2</sub>H<sub>2</sub> patients had side effects in the first three months of treatment, 20 per cent, 17 per cent, 13 per cent and 27 per cent respectively having major departure of 7 days or more from the prescribed chemotherapy. From four to twelve months 21 per cent of the P, 11 per cent of the E, 10 per cent of the R and 27 per cent of the S<sub>2</sub>H<sub>2</sub> patients had side effects, 9 per cent, 5 per cent, 1 per cent and 8 per cent respectively having departure from chemotherapy.

In the first 3 months vestibular side effects, invariably due to streptomycin resulted in major departure from chemotherapy in 9 per cent of the P, 11 per cent of the E, 6 per cent of the R and 13 per cent of S<sub>2</sub>H<sub>2</sub> patients had gastric side effects in 5 per cent, 1 per cent, 3

per cent and 7 per cent respectively. Cutaneous reactions almost always attributed to streptomycin or PAS were responsible for major departures from chemotherapy in 10 per cent of P, 5 per cent of E, 4 per cent of R and 12 per cent of S<sub>2</sub>H<sub>2</sub> patients in the first 3 months. Side effects continued to occur particularly in the P and S<sub>2</sub>H<sub>2</sub> regimens from 4 to 12 months. In both periods side effects were due to streptomycin or PAS and there were few reactions to ethambutol, rifampicin or isoniazid. In the first 3 months vestibular side effects were more in all regimens in older patients and gastric effects in the P and S<sub>2</sub>H<sub>2</sub> series. During the subsequent 9 months, the higher frequency of gastric side effect in the P series and vestibular side effects in the S<sub>2</sub>H<sub>2</sub> series.

Thirty six (7.7 per cent) had resistance to 1 more of the 3 standard drugs by either the standard MIC or proportion test definition in at least one of their pre-treatment cultures.

H.B.D.